



EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. Scientific Opinion on the public health risks of table eggs due to deterioration and development of pathogens

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SCIENTIFIC OPINION

Scientific Opinion on the public health risks of table eggs due to deterioration and development of pathogens¹

EFSA Panel on Biological Hazards (BIOHAZ)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Salmonella Enteritidis is considered the only pathogen currently posing a major risk of egg-borne diseases in the European Union (EU). The possible impact of extending the shelf-life of eggs on the risk to consumers posed by *S. Enteritidis* was estimated by applying a quantitative model and comparing the actual situation regarding the storage of eggs in the EU with different possible scenarios combined, considering the prolongation of the best-before and the sell-by date from 7 to a maximum of 70 days. Extending the sell-by date by one week (from 21 to 28 days), but leaving the best-before date unchanged, is estimated to result in a relative risk of illness of 1.4 and 1.5 for uncooked and lightly cooked egg meals respectively, compared to the current situation. If the best-before date is also extended by one week (from 28 to 35 days), the relative risk would be 1.6 and 1.7. In the worst case scenario considered (sell-by date of 42 days, best before date of 70 days), such figures would be 2.9 and 3.5. It should be noted that the absolute risk is greater for uncooked meals compared to lightly cooked meals. An effective way to minimise any increase in risk during extended storage is to keep the eggs refrigerated both at retail and the household. Regarding egg spoilage, such events strongly depend on the hygienic conditions of egg production and practices of egg handling, including storage times and temperatures. Finally, the impact of the prolongation of storage time on the quality criteria for eggs (3-hydroxybutyric acid and lactic acid) destined for manufacturing of egg products is considered negligible.

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KEY WORDS

table eggs, sell-by-date, best-before date, *Salmonella Enteritidis*, storage time

¹ On request from the European Commission, Question No EFSA-Q-2013-00400, adopted on 10 July 2014.

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ Panel) was asked by the European Food Safety Authority to deliver a scientific opinion on the public health risks of table eggs due to deterioration and development of pathogens.

Specifically, EFSA was asked to assess: (i) the public health risk posed by relevant pathogens and in particular by *Salmonella* in the consumption and handling of table eggs (Term of Reference, or ToR1), (ii) the public health risk deriving from deterioration (ToR2) and (iii) possible consequences for public health of an extended shelf-life of table eggs for the specific freshness criteria for egg products as laid out in the hygiene package (ToR3).

In order to answer the first ToR, the BIOHAZ Panel based its conclusions on the results of a quantitative model, aimed at describing the behaviour of *Salmonella* Enteritidis following vertical transmission, since this serovar is recognised to be the major pathogen related to egg-borne disease because of its ability to contaminate the interior of intact eggs during their formation within the body of an infected hen. The possible impact of extending the shelf-life of eggs on other serovars of *Salmonella*, as well as on other pathogens, is evaluated in a qualitative manner, with no individual serovar other than *S. Enteritidis* currently posing a major risk of egg-borne salmonellosis in the European Union (EU), although *S. Typhimurium* has been associated with relatively small outbreaks due to duck eggs. Trans-shell contamination (i.e. secondary contamination) is also discussed, but the impact of a prolongation of the shelf life on trans-shell contamination under modern conditions of hygienic egg production is considered minor. The role of external contamination of the shell in public health risk is currently uncertain due to lack of data.

The quantitative model is based on an existing model commissioned by the Australian Egg Corporation Limited (AECL), which was modified in order to make it more relevant to the European situation. The quantitative model excludes all stages before lay, since the Panel considered that possible changes in egg shelf life will only impact on the behaviour of *S. Enteritidis* from the point of lay onward, as all previous stages remain unaffected by changes in storage time. The model therefore does not include the farm phase, and prevalence inputs for the model are derived from a combination of the estimated rate of egg contents contamination from *S. Enteritidis*-positive flocks and the within-flock prevalence, as reported in the literature, and the estimated prevalence of *S. Enteritidis* in laying flocks in the EU according to harmonised monitoring data. A baseline scenario has been defined according to the current situation regarding sell-by and best-before dates in the EU. Alternative scenarios, changing the time and temperature of storage in different phases (retail and household), were used to assess the impact of possible changes in current storage practices. The model considers only *Gallus gallus* eggs, and those from other species are dealt with in a qualitative way because of lack of data, but their role is assumed to be minor as the market for such eggs is small. Taking into consideration the importance of pooling of eggs as a risk factor for foodborne outbreaks, both household and food service and institutional settings are modelled in order to assess the impact of any changes in storage conditions.

According to the results of the model, prolongation of the storage time for table eggs results in an increase in the number of illnesses per million servings, except when eggs are well-cooked. The magnitude of this increase depends on the additional time of storage that the eggs spend at both retail and in households. An effective way to minimise any increase in risk during extended storage is to keep the eggs refrigerated at both retail and the household.

Extending the sell-by date by one week (from 21 to 28 days), but leaving best-before date unchanged, is estimated to result in a relative risk of illness of 1.4 and 1.5 for uncooked and lightly cooked egg meals respectively, when compared to the baseline. If the best-before date is also extended by one week (from 28 to 35 days), the relative risk would be 1.6 and 1.7. In the worst case scenario considered in this assessment (sell-by date of 42 days, best before date of 70 days), such figures would

be 2.9 and 3.5. It should be noted however that the absolute risk is greater for uncooked meals compared to lightly cooked meals.

The implementation of refrigeration as currently used in the EU during the retail stage (i.e. with temperatures assumed to range from 0 to 12 °C) limits to some extent this increase in the risk. The risk is reduced in the case of a prolongation of up to three weeks in the sell-by date, and also of one or two weeks of the best-before date for a sell-by date of 35 and 28 days respectively, if refrigeration is applied during storage in all retail establishments. If the sell-by date or the best-before date are prolonged beyond three weeks, the risk estimates are greater, even if refrigeration at retail is applied, assuming that the proportion of consumers who do not store their eggs under refrigeration remains unchanged.

As far as pooling at household level as well as in catering/food service and institutional settings is concerned, the relative risk of illness estimates show an increase of the risk with storage time, similar to that observed for individual eggs. It should be noted that the risk arising from pooled eggs would increase if the time or the temperature of storage of the pool increases significantly (i.e. under poor food hygiene practices).

The uncertainties associated to the assumptions made and to the data used in this assessment will affect the absolute estimates of the risk. The combined effect of all uncertainties is difficult to measure, but nevertheless the absolute risk estimates should be used with caution. The relative risk estimates are less influenced by uncertainty associated to both the baseline and the alternative scenarios

For answering ToR 2, a review of the organisms involved in spoilage of hens' eggs was conducted. During storage, gaseous exchange between the egg content and the atmosphere, as well as exchanges of water and minerals between egg albumen and egg yolk lead to decreasing egg albumen defence mechanisms and weakening of the vitelline membrane, increasing the risk of bacterial invasion of the egg internal compartments. There is a clear deleterious effect of high storage temperatures and/or long storage periods on the internal egg quality and the rate of development of macroscopic changes in table eggs, particularly if eggs are contaminated by spoilage bacteria. While the effect of the storage temperature on the level of surface bacteria is variable according to a combination of conditions, temperature, time, and humidity are crucial parameters involved in the decrease of egg quality throughout storage, increasing the risk of trans-shell microbial invasion. Storage at chilled temperatures therefore helps maintain overall physicochemical and microbiological quality of eggs. Egg spoilage events strongly depend on the hygienic conditions of egg production and practices of egg handling, including also storage times and temperatures. It should be noted that the characteristics of egg spoilage are mainly the results of macroscopic changes in their odour and/or colour or viscosity, which would prevent the egg being used for food products.

Finally, in the answer to ToR 3, the Panel assessed the impact on, and relevance of, the quality criteria for eggs destined for manufacturing of egg products (as defined in Regulation No (EC) 853/2004: (i) the concentration of 3-hydroxybutyric acid and (ii) the lactic acid content of the raw material used to manufacture egg products.

Of the two currently-recommended indicators, 3-hydroxybutyric acid is exclusively related to detection of the use of embryonated eggs, and is therefore related more to fraudulent practice than to microbial growth or the conditions of storage, as its concentration is not influenced by storage time if eggs are not fertile. Even if present at trace levels in infertile eggs, its concentration does not increase during the storage, regardless of the storage conditions. Lactic acid is recognised as an indicator of microbial degradation of table eggs. It is present in the egg due both to the development of the embryo in fertile eggs and to microbial growth. The latter will be affected by the conditions of storage, and the concentration of lactic acid increases with egg storage time, but the levels of lactic acid found in eggs that have past their shelf life are less than those found in some other commonly consumed food products, such as fermented milk products (e.g. yogurt or cheese). Microbiological criteria are set in

European legislation for egg products, i.e. a food safety criterion for *Salmonella* and a process hygiene criterion for Enterobacteriaceae at the end of the process of egg product manufacturing, and these provide a suitable indication of microbial contamination.

The Panel recommends conducting further studies on risk assessment exploring the effect of different temperatures of storage of eggs on the risk posed by egg borne pathogens such as *S. Enteritidis*, and to investigate the occurrence and control of microorganisms during industrial production of egg products, including pasteurisation, if the storage of eggs is prolonged, and suggests a re-evaluation of the current chemical indicators, considering the possibility of using more relevant ones.

Some knowledge gaps are identified, concerning the production and consumption of eggs from avian species other than chickens, the potential for growth of *Salmonella* in relation to the breakdown of the yolk membrane in eggs from current large-scale production, and the occurrence and control of microorganisms in industrial manufacture of egg products.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Pre-packed foods, with few exceptions, must bear a date of minimum durability (best before date) or a use by date. EU law⁴ specifies that the “best before” date should be replaced by a “Use By date” when, from a microbiological point of view, a food is highly perishable and is therefore likely after a short period to constitute an immediate danger to human health. In addition, the new Regulation on the provision of food information to consumers⁵, clarifies in Article 24 that after the “Use By date” a food shall be deemed to be unsafe in accordance with Article 14(2) to (5) of Regulation (EC) No 178/2002⁶.

On the contrary, the “Best-Before date” relates to the date until when the food retains its specific properties when properly stored. Hence, even after this date, a food may still be consumed and sold, if the food business operator can assure that the food still meets all food law requirements.

The “Best-Before date” applicable to eggs marketed as class “A/Fresh” is fixed in the Regulation 589/2008 at 28 days from laying, the period during which an egg properly stored normally retains its specific quality properties. This is linked to quality parameters and not to any *Salmonella* criterion for eggs or the *Salmonella* situation in flocks of laying hens.

A “Sell-By date” is fixed at 21 days within the Hygiene Regulation in order not to mislead the consumer and to maintain a reasonable, harmonised shelf-life. This means that table eggs must be delivered to the consumers within a maximum time limit of 21 days after laying. The eggs that are removed from the shelves for having gone beyond the “Sell-by date” may be diverted by retailers to the egg processing industry.

For egg products, freshness criteria such as thresholds for lactic and butyric acid have in any case to be met according to Regulation (No) 853/2004⁷. A longer shelf-life of eggs might lead to negative consequences on the quality of such products.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is asked to issue a scientific opinion on the public health risks of eggs due to deterioration and development of pathogens and in particular, to:

- assess the public health risk posed by relevant pathogens and in particular by *Salmonella* in the consumption and handling of table eggs and to quantify the relevance of underlying factors such as the observed flock prevalence in a Member State/region, the period of time between laying and consumption, the cooling and storage conditions, consumer behaviour (e.g. cooking, cross-contamination) etc.;
- assess the public health risk deriving from deterioration taking into account the underlying risk factors such as hygiene, the cooling and storage conditions in the period between laying and consumption, consumer behaviour, etc.;
- assess possible consequences for public health of an extended shelf-life (after the sell-by date) of table eggs for the specific freshness criteria for egg products as laid out in the hygiene package.

⁴ Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. OJ L 109, 6.5.2000, p.29.

⁵ Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004, OJ L 304, 22.11.2011, p.18.

⁶ Regulation (EU) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, p. 31.

⁷ Point 4 of Chapter II of Section X of Annex III to Regulation (EC) No 853/2004.

ASSESSMENT

1. Introduction and approach taken

Eggs are very important and complete foods not only for their nutritional aspects (e.g. high-quality proteins, vitamins A, B12, D and E), but also for their functional properties, i.e. the coagulant capacity of proteins, the foaming capacity of albumen proteins, the emulsifying capacity of the yolk, etc. These properties are used in different ways to produce and enrich many types of foods (e.g. bakery products including pastries, meat pies, sauces and dressings, sweets and pasta) and in several (homemade) dishes (e.g. mayonnaise, custard and ice cream). Eggs are often used raw or only lightly heat treated in such products. Eggs and egg products are used in many different locations (domestic kitchens, restaurants and catering outlets, food industry establishments) and can be vehicles for food-borne hazards. The storage conditions of the eggs, such as temperature and time, have an impact on the risk derived from these hazards.

The present scientific opinion deals with the public health risks of eggs due to deterioration and development of pathogens. In accordance with the terms of reference (ToRs) provided by the European Commission, the European Food Safety Authority (EFSA) was asked to assess the public health risk posed by relevant pathogens, and in particular by *Salmonella*, in the consumption and handling of table eggs and to quantify the relevance of underlying factors such as the observed flock prevalence in a Member State (MS)/region, the period of time between laying and consumption, the cooling and storage conditions, consumer behaviour (e.g. cooking, cross-contamination), etc. EFSA was also asked to assess the public health risk arising from deterioration taking into account the underlying risk factors such as hygiene, the cooling and storage conditions in the period between laying and consumption, consumer behaviour, etc. In addition, EFSA was requested to assess the possible consequences for public health of an extended shelf life (beyond the sell-by date) of table eggs for the specific freshness criteria for egg products laid out in the hygiene package.

Two EFSA scientific opinions have been published in recent years dealing with hens' eggs: microbiological risks on washing (EFSA, 2005) and cooling of table eggs (EFSA, 2009b). In addition, EFSA published a scientific opinion on an estimate of the public health impact of setting a new target for the reduction of *Salmonella* in laying hens (EFSA BIOHAZ Panel, 2010a).

Approach followed to address the terms of reference

In order to address the first ToR, the Panel agreed to base its conclusions on the results of a quantitative model, aimed at describing the behaviour of *S. enterica* subsp. *enterica* serovar Enteritidis following vertical transmission, as this serovar is recognised as the major pathogen related to egg-borne disease. The possible impact of extending the shelf life of eggs on other serovars of *Salmonella*, as well as on other pathogens, is evaluated in a qualitative manner. Trans-shell contamination (i.e. secondary contamination) is also discussed in the body of the opinion.

The quantitative model is based on an existing model commissioned by the Australian Egg Corporation Limited (AECL) (Thomas et al., 2006) that was modified in order to make it more relevant to the European situation and more suitable to address ToR 1, as described in Section 13.2. Data used to implement the model are also described in that section. The quantitative model excludes all stages before lay, as the Panel considered that possible changes in egg shelf life will impact on the behaviour of *S. Enteritidis* only from that moment onwards, as all previous steps will remain unchanged. The model therefore does not include the farm phase, and prevalence inputs for the model are a combination of the estimated rate of contamination of egg contents from *S. Enteritidis*-positive flocks as reported in the literature, and the estimated prevalence of *S. Enteritidis* in laying flocks in the European Union (EU) derived from harmonised monitoring data. The consumer phase is taken into account, as it can modulate the risk of disease owing to the consumption of eggs that have been stored for different periods at different temperatures or prepared in different ways. A baseline scenario has been defined based on expert opinion and on the answers collected through questionnaires that were sent to the MSs and to the associations of egg producers in the EU countries, in order to get

information on current practices in the egg production chain. Alternative scenarios, changing the time and temperature of storage in different phases (retail and household), were used to assess the impact of possible changes in current practices. The model considers only *Gallus gallus* eggs, and those from other species are dealt with in a qualitative way.

Taking into consideration the importance of pooling of eggs as a risk factor for foodborne outbreaks, both household and food service and institutional settings are modelled in order to assess the impact of any changes in shelf life conditions. Different subgroups of the consumer population, including vulnerable groups, are considered in the model, as it applies a dose–response model based on outbreak data that includes both healthy and susceptible individuals.

In addressing both ToR 1 and 2, only class A table eggs are considered.

To address ToR 2, a review of the organisms involved in the spoilage of hen's eggs was conducted, taking into account the fact that spoilage bacteria can affect the growth of *Salmonella* and other pathogens as well as lead to rejection of eggs as 'unacceptable', and therefore they have to be considered when the risk related to pathogen survival and/or growth is estimated.

Finally, in the response to ToR 3, the Panel assessed the impact on, and relevance of, the quality criteria for eggs destined for manufacturing egg products (as defined in Regulation (No) 853/2004: (i) the concentration of 3-hydroxybutyric acid must not exceed 10 mg/kg in the dry matter of the unmodified egg product; (ii) the lactic acid content of the raw material used to manufacture egg products must not exceed 1 g/kg of dry matter) depending on changes in the duration and temperature of storage.

2. Egg and egg products: regulatory framework, production and consumption in the EU

2.1. Current regulatory situation for the storage of table eggs throughout the food chain

In Regulation (EC) No 589/2008⁸, 'eggs' are defined as eggs in shell — other than broken, incubated or cooked eggs — that are produced by hens of the species *Gallus gallus* and are fit for direct human consumption or for the preparation of egg products. Even if eggs used for these purposes are from laying hens, other birds (e.g. ducks, quails) can also produce eggs for human consumption.

In the EU, since 1 January 2004 only two classes of eggs, A and B, can be sold. The quality characteristics of class A eggs are defined in this Regulation as follows:

- shell and cuticle: normal shape, clean and undamaged;
- air space: height not exceeding 6 mm, stationary; however, for eggs to be marketed as 'extra', height may not exceed 4 mm;
- yolk: visible on candling as a shadow only, without clearly discernible outline, slightly mobile upon turning the egg, and returning to a central position;
- white: clear, translucent;
- germ: imperceptible development;
- foreign matter: not permissible;
- foreign smell: not permissible.

Class B eggs shall be eggs which do not meet these quality characteristics and class A eggs which no longer have those characteristics may be downgraded to class B. These eggs can be only sold to industry, not as table eggs, and are required to be marked as such.

⁸ Commission Regulation (EC) No 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs. OJ L 163, 24.6.2008, p. 6-23.

Eggs that are not sold as class A table eggs, because of either quality or production issues, can be sent for further processing, which usually aims to prolong the shelf life of the egg contents without compromising the appreciated functional properties of its components. According to these regulations, class A eggs can be considered as equivalent to 'table eggs' (only these eggs are fit for sale to the end consumer at retail).

According to the Codex Alimentarius Code of hygienic practice for eggs and egg products (CAC/RCP 15—1976), a table egg is 'an egg destined to be sold to the end consumer in its shell and without having received any treatment significantly modifying its properties', and egg product is 'all, or a portion of, the contents found inside eggs separated from the shell, with or without added ingredients, intended for human consumption'. The 'best-before date' applicable to eggs marketed as class A is fixed in Regulation (EC) No 589/2008 at 28 days from laying, the period during which an egg properly stored normally retains the above-mentioned quality characteristics. A 'sell-by date' is fixed at 21 days within Regulation (EC) No 853/2004⁹ in order not to mislead the consumer and to maintain a reasonable, harmonised shelf-life. This means that table eggs must be delivered to the consumers within a maximum time limit of 21 days after laying. The eggs that are removed from the shelves because they have gone beyond the 'sell-by date' may be diverted by retailers to the egg-processing industry. The words 'extra' or 'extra fresh' may be used as an additional indication of quality on packs containing class A eggs until the ninth day after they were laid.

The provisions regarding egg product processing are laid down in Chapter X, Annex III, of Regulation (EC) No 853/2004. Council Directive 89/437/EEC of 20 June 1989¹⁰ establishes the requirements for hygiene and health issues relating to the production and marketing of egg products intended for direct human consumption or as ingredient in foodstuffs.

Regulation (EC) No 853/2004 also defines requirements for the manufacture of egg products. For egg products, freshness criteria are defined:

- The concentration of 3-hydroxybutyric acid must not exceed 10 mg/kg in the dry matter of the unmodified egg product;
- The lactic acid content of raw material used to manufacture egg products must not exceed 1 g/kg of dry matter. However, for fermented products, this value must be the one recorded before the fermentation process.

Regulation (EC) No 2073/2005¹¹ defines the following microbiological criteria in relation to eggs and egg products:

- Food safety criteria (absence in 25 g) for *Salmonella* in 'Egg products, excluding products where the manufacturing process or the composition of the product will help to minimise the *Salmonella* risk' for products placed on the market during their shelf life;
- Food safety criteria (absence in 25 g) for *Salmonella* in 'Ready-to-eat foods containing raw egg, excluding products where the manufacturing process or the composition of the product will help to minimise the *Salmonella* risk' for products placed on the market during their shelf life;
- Process hygiene criteria for Enterobacteriaceae in egg products apply at the end of the manufacturing process; in the case of unsatisfactory results the actions defined are checks on the efficiency of the heat treatment and prevention of recontamination.

⁹ Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 55-205.

¹⁰ Council Directive 89/437/EEC of 20 June 1989 on hygiene and health problems affecting the production and the placing on the market of egg products. OJ L 212, 22.7.1989, p. 87-100.

¹¹ Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-29.

On farm, national legislation or guidance in MSs may apply, for example a maximum of three days in the United Kingdom. Article 6 of Regulation (EC) No 589/2008 states that eggs shall be graded, marked and packed within 10 days of laying.

According to Regulation (EC) No 853/2004¹², eggs must be stored and transported until sale to the final consumer at a temperature, preferably constant, that is best suited to ensuring optimal conservation of their hygiene properties, unless the Competent Authority imposes national temperature requirements for egg storage facilities and for vehicles transporting eggs between such storage facilities. In addition, Regulation 589/2008 establishes that *'eggs shall not be treated for preservation or chilled in premises or plants where the temperature is artificially maintained at less than 5 °C. However, eggs which have been kept at a temperature below 5 °C during transport for not more than 24 h or on retail premises or in annexes thereto for not more than 72 h shall not be considered as chilled.'* In many European countries there are no specific legislative requirements regarding time/temperature conditions during storage. Several countries establish a maximum temperature of 18 °C either throughout the whole egg chain or in certain stages. In one MS, shell eggs to be sold commercially must be kept at a temperature of between 5 °C and 8 °C from the 18th day after laying onwards including during transportation and throughout storage. In another MS, the temperature is restricted to 12 °C throughout the whole egg chain. Some countries have issued diverse guidelines with regard to the handling and storage of eggs on farm, during transport, at retail, at household level and in catering and institutional settings, such as time/temperature combinations, relative humidity, restrictions on the use of fresh eggs for specific populations or labelling. Producers or distributors may include instructions on the label of fresh egg packages indicating that eggs should be kept refrigerated at household level after buying, in accordance with Article 3(1)(6) of Directive 2000/13/EC.

2.2. Structure of production

Grand parent and parent flocks are genetically selected on various criteria applicable for the parent and for the progeny (hatchability, number of eggs produced, colour of shell, suitability of progeny for the type of production (colony cages, alternative systems)). Aspects such as bone strength and resistance to certain diseases are also often subject to selection. Fertile eggs from these flocks are incubated and hatched under very good sanitary conditions to produce grandparent birds that produce parent birds which then produce the commercial generation of laying hens. This multiplication pyramid means that one elite breeding hen can be responsible for up to 300 000 commercial laying birds and over 90 million eggs. Female day-old laying hen chicks are selected, vaccinated and transferred to the rearing farm. During the rearing period, pullets are housed in floor-based systems or in cages for 16 to 18 weeks, then transferred to the laying phase; this transfer is an important stress factor for the pullets.

During the laying period, which is approximately 44 to 60 weeks in most cases, production of eggs increases progressively to reach peak production (0.9 eggs per day) 6 to 8 weeks after the onset of lay. The quality of the egg (colour, weight, size and conformation) and eggshell also increases progressively during these first weeks of lay, and then deteriorates towards the end of lay, although egg size may continue to increase. At the end of the laying period, a moulting procedure is occasionally used to stimulate a new period of lay by means of nutrient and light restriction. This is a stressful process that can increase the risk of *Salmonella* shedding by latent carrier birds. More commonly, laying hens are transported to the slaughterhouse to be slaughtered after a single laying period.

A basic representation of the egg production chain, from breeding flock to egg collection, is shown in Figure 1.

¹² Amended by Regulation (EC) No 1020/2008.

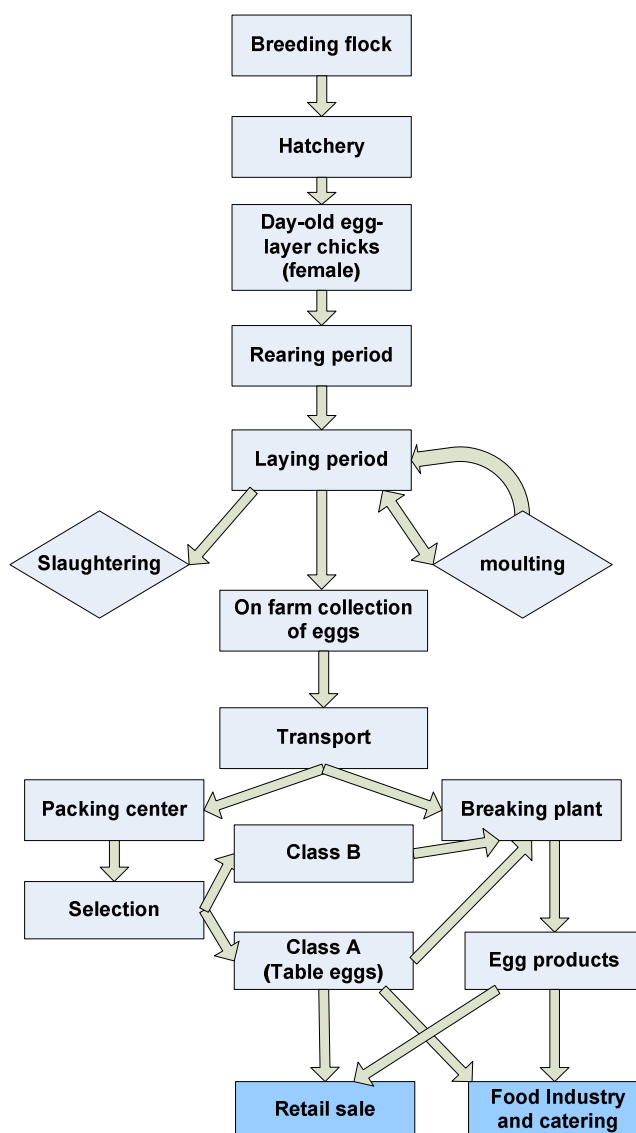


Figure 1: The basic egg production chain, from breeding flock to retail (adapted from EFSA BIOHAZ Panel (2010a)).

During the laying period, eggs are collected every day, sometimes several times a day, using automated belts transporting the eggs from auto-nests or cage egg belts to a collecting room. On small farms, eggs may be collected manually into trays held on trolleys or buggies. Eggs are selected and stored in a storage area, which may sometimes be chilled, before transfer to the packing centre, which may be on the farm or a separate central facility, or to an egg processing (breaking) plant (see Figure 1 above).

Following the new EU Regulation concerning the minimum standards for the protection of laying hens (Council Directive 1999/74/EU¹³) banning conventional ‘battery’ cage production, alternative systems have replaced conventional cages in all MSs. This involves the use of enriched colony cages housing up to 80 birds per large cage area following new European standards, barn and aviary systems, which may be of multi-tier construction, or free range and organic production with outdoor access (Dekker et al., 2011). The choice of the type of production is clearly different between MSs, and each system has different advantages and disadvantages in terms of bird welfare and biological risk (Holt et al., 2011;

¹³ Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. OJ L 203, 3.8.1999, p. 53-57.

Lay et al., 2011). The regulatory requirements have resulted in many small cage layer units going out of business.

2.3. European Union egg production

In 2011, egg production in the EU-27 was estimated at 7.12 million tonnes (equivalent to over 100 billion eggs). Almost 75 % of these eggs were produced in seven MSs (France, Germany, Italy, Spain, the United Kingdom, the Netherlands and Poland). Overall production could be considered to be stable (- 0.1 % compared with 2007), but changes in production vary between MSs (e.g. - 5.6 % in Spain and + 5.5 % in the United Kingdom) (Agriculture in the European Union, statistical and economic information. Report 2012¹⁴). Laying hen population figures reflect those of egg production (see Figure 2).

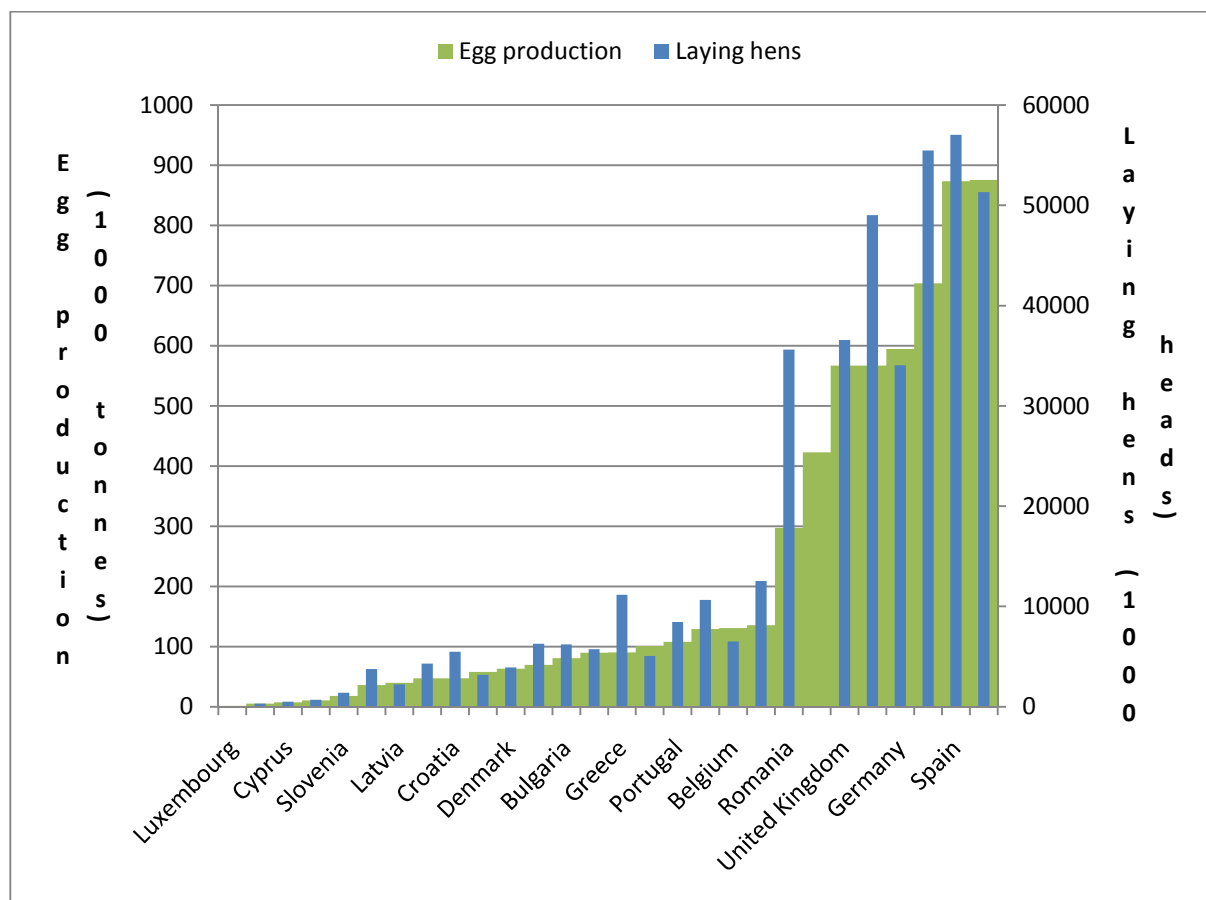


Figure 2: Population of laying hens and production of table eggs in EU Member States, reported from 2003 to 2010 depending on Member State. Data on the population of laying hens were not available for the Netherlands (Statistical database of EUROSTAT¹⁵, extracted 16 July 2013)

2.4. Egg products

2.4.1. Introduction

The European egg product industry mainly purchases eggs directly from farms or from packing centres. Regulation (EC) No 853/2004 also authorises the processing of eggs downgraded by packing centres, including cracked eggs or ones produced on farms contaminated with *Salmonella*. Cracked eggs and eggs provided by contaminated farms must be broken and pasteurised upon arrival in egg-processing plants. Information received from the egg industry indicates that in most countries table

¹⁴ Available at http://ec.europa.eu/agriculture/statistics/agricultural/2012/pdf/full-report_en.pdf, accessed 17 June 2014.

¹⁵ <http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/>

eggs that have reached their 'sell-by date' at retail are not diverted to egg products, as this practice is rarely economically viable. When such practices do occur, they are small in scale. According to the current legislation, there is no 'best before date' for eggs destined for the production of egg products.

Commission Regulation (EC) No 853/2004 defines egg products as 'processed products resulting from the processing of eggs, or of various components and mixtures of eggs, or from the further processing of such processed products'. Processing consists of 'any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes', as described in Commission Regulation EC No 852/2004¹⁶. Egg products from 'first processing' are defined as resulting from the breaking of table eggs, giving rise to the recovery of whole egg or separated egg yolk and egg white, with the possible addition of salt, sugar and hydrocolloids (Lechevalier et al., 2011). They are mainly delivered in the form of refrigerated liquid egg, but also as frozen or dried powder products. European data are not available, but in France, which is the largest European producer of egg products, around 93 % of the total volume was produced in the form of liquid egg products, while dried egg products represent only 7 % of the French production in 2012 (ITAVI, 2013).

Egg products are widely used for various food applications, suitable for artisans, catering and as ingredients for the food industry, being used in sauces, pasta, biscuits, cakes, processed meats, fish products, wine products, ice creams and refrigerated desserts. In some countries, 'first processing' egg products may also be available for consumers, e.g. as pasteurised liquid egg for body-builders or home cooking.

In addition to egg products from 'first processing', which are by far the predominant products of the egg-processing industry, 'speciality' egg products are also manufactured. These products result from the cooking of either table eggs or formulated egg products (whipped egg whites, poached eggs, scrambled eggs, hard-boiled eggs, pickled eggs, fried eggs or omelettes). They are sold either directly to consumers or through mass catering.

2.4.2. Industrial production of egg products from 'first processing'

Eggs used for egg product manufacturing are often by-products of those graded as table eggs that do not meet the required specifications. These eggs are out-of-grade or downgraded ones, including floor and cracked eggs that may present a greater risk of contamination. Some egg production farms are also especially devoted to the egg product industry. Small laying farms sometimes invest in small breaking units in order to utilise downgraded eggs. These farms mainly produce pasteurised liquid whole egg intended for a local market (Lechevalier et al., 2011). Specific industrial units used by the largest egg product companies are dedicated to egg breaking, cracking and formulation. For some units, established within dedicated laying farms, the eggs are transformed without being previously packed. For the other units, eggs can be obtained either from packaging centres or from farms under contract (Lechevalier et al., 2011). A schematic representation of this process can be found in Figure 3.

¹⁶ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 131, 30.4.2004, p. 1-54.

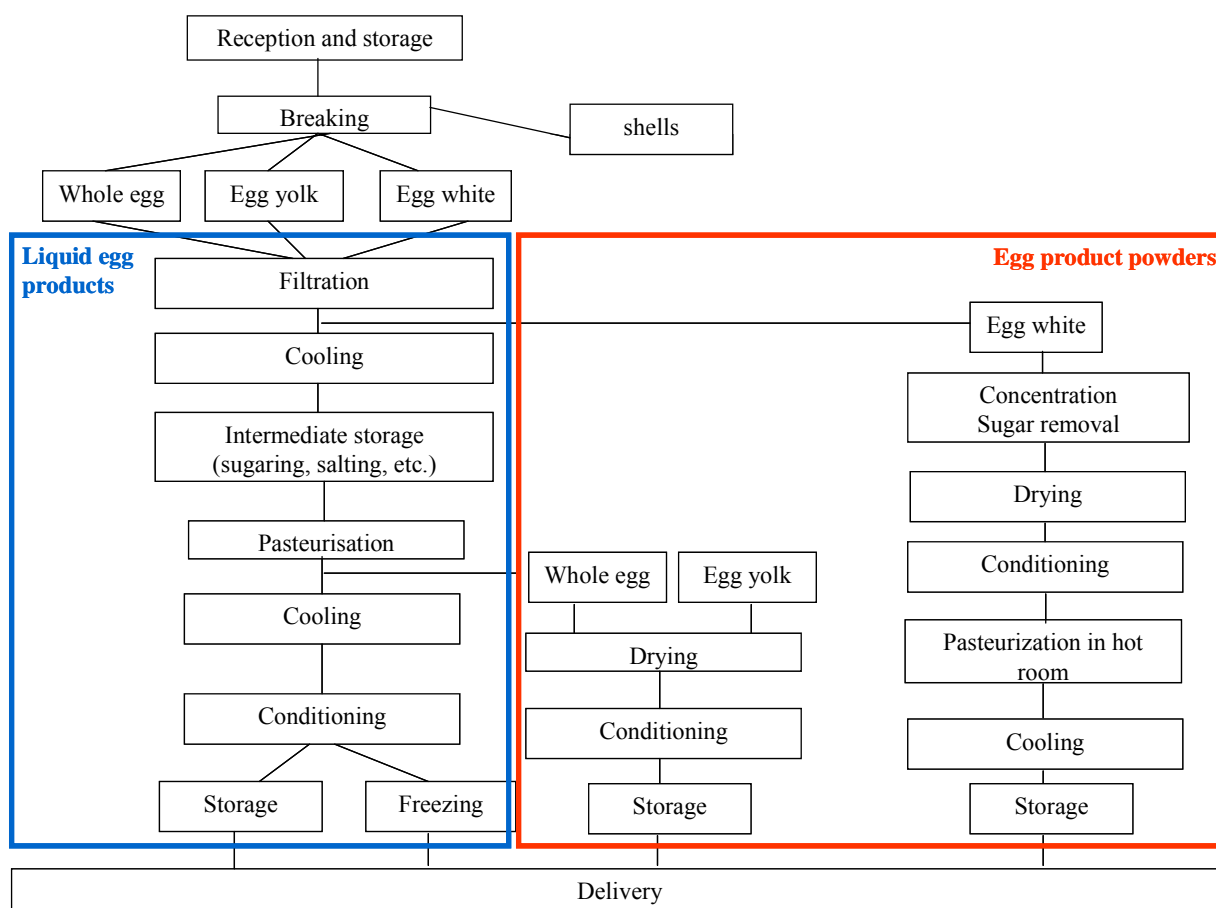


Figure 3: The major steps in the industrial production of egg products from ‘first processing’

The provisions ensuring the production of safe egg products are described in the following paragraphs.

2.4.2.1. Provisions regarding egg quality

Upstream of the transformation process, egg quality is first evaluated by the examination of the external appearance of the egg, and of the quality of the packaging. The eggs should be packaged on plastic trays that can be washed and recycled under controlled conditions of hygiene, temperature and humidity. Eggshells must be fully developed and contain no breaks. Cracked eggs may be used if they are delivered directly to egg product manufacture, where they must be broken as soon as possible owing to their greater susceptibility to microbial penetration. Assessment of egg freshness (see Section 10) can be done before egg breaking depending on the type and final use of the processed egg product (whole egg, egg white or egg yolk intended for cooked or uncooked foods). Regulation (EC) No 853/2004 authorizes the processing of eggs downgraded by packing centres (misshapen, dirty or broken eggs; eggs with cracked eggshells) or even from farms contaminated by *Salmonella*, as long as the eggs are broken upon receipt and the egg products are heat-treated immediately. Downgrading may vary from 1 % or 2 % to 35 % depending on the age of the hens and on the extent of damage to the eggs throughout the logistic chain (Travel and Nys, 2011). Egg products from such consignments must be quickly pasteurised and used for low risk applications from a microbiological point of view.

2.4.2.2. Provisions regarding egg storage

Before the breaking stage, eggs should be stored in a secure room that is separated from the product line at a controlled temperature (around 15 °C) or under positive chilled temperatures (4 °C), depending on the storage period. Proper storage conditions, especially with regard to the control of temperature, humidity and hygiene, are essential to limit the penetration, migration and multiplication of microorganisms (see Section 8). It should be noted that the rate of degradation of the vitelline

membrane is reduced by the storage of eggs at refrigeration temperatures (see Section 10.5). The effect of long term storage of shell eggs on egg product microbiological quality has not been fully reported in the literature, apart from the suggestion that egg white pH increases during storage may have consequences for the efficiency of pasteurisation by modifying the thermal sensitivity of bacteria (Baron and Jan, 2011; Lechevalier et al., 2011), including *Salmonella* (Froning et al., 2002), and that the use of fresh or refrigerated eggs ensures the best separation of egg white from egg yolks, consequently decreasing the microbial risk of egg white contamination by pathogenic flora, including *Salmonella* and *Listeria monocytogenes*.

2.4.2.3. Provisions regarding washing of eggs

Washed table eggs may only be marketed in the MSs in which an authorisation for such practice has been issued. In contrast to eggs marketed as table eggs from chickens, egg washing before processing is permitted in the EU (Regulation (EC) No 853/2004, Regulation (EC) No 589/2008), provided that the eggs are immediately broken after washing. Advantages and disadvantages of egg washing from a food safety point of view are discussed elsewhere (EFSA, 2005). Egg washing may be implemented for specific applications, such as those involving the use of egg products for the preparation of foods that can present a high risk from a microbiological point of view. For economic and technical purposes and to avoid problems associated with ineffective washing, egg washing is not a current practice in European egg-processing plants. The egg washing process needs to be improved, particularly regarding the prevention of rapid fouling of the washing machines by egg debris, which currently limits their effectiveness.

2.4.2.4. Provisions regarding egg breaking

The eggs must be broken individually, so breaking by centrifugation or crushing, which was previously allowed, is now forbidden due to the high microbiological risk that this represents (Cassin, 2010). Egg breaking should be carried out in a separate room from where eggs are stored. During the breaking operation, the eggs must be clean and dry. Legally, it is possible to break eggs of species other than chicken. Duck, turkey and guinea fowl eggs must, for health purposes, be handled separately and broken after complete cleaning and disinfection of the equipment. This possibility is, however, hypothetical, as there is no relevant market for such products.

The breaking machines are designed to break the eggs, to remove the eggshells and to separate egg yolk from egg white. The contact of shells with the breaking instruments and surfaces results in systematic contamination of the egg contents. Moreover, once broken, the egg loses much of its natural defences (see Section 9.1). The quality of the separation depends on the storage temperature of the eggs (better in chilled eggs), on their freshness (regarding the integrity of the vitelline membrane), on the type of machine and on the rate of breakage. Egg freshness is a crucial parameter for egg white product manufacturing. The antibacterial properties of egg white are reduced in the presence, even at trace levels, of egg yolk, which may provide sufficient iron and nutrients for bacterial growth (see Sections 9 and 10). Moreover, the absence of egg yolk is recommended for optimising the foaming properties of egg white, while it has less impact on its gelling properties (see Appendix A). In order to ensure proper egg white separation and stabilisation, the eggs used can therefore be sorted according to their freshness.

2.4.2.5. Provisions regarding the removal of eggshells and chalazae

The shells are quickly evacuated to minimise their contact with the egg products. They are crushed and centrifuged in a separate room. The resulting highly contaminated by-products (liquid waste and crushed shells) should be removed as soon as possible from the manufacturing process.

Shell debris and chalazae remaining in the egg product are immediately removed by sieving and filtration. The target is less than 100 mg shell/kg of liquid product, as stated in Regulation (EC) No 853/2004. This step also provides complete homogenisation of the egg products.

2.4.2.6. Provisions regarding the storage period before stabilisation

After filtration, the egg products are pasteurised or chilled as rapidly as possible, stored at refrigeration temperatures for no more than 48 hours (Regulation (EC) No 853/2004) and pasteurised, except for egg white intended for drying (the pasteurisation then takes place after drying), and for salted egg yolk (because refrigeration would increase the egg yolk viscosity and would not allow the liquid to be pumped through the pipes of the pasteuriser) (Cassin, 2010). In addition to pasteurisation, other industrial practices contribute to the microbial stabilisation of the egg products, such as concentration, drying, freezing, sugaring and/or salting.

2.4.2.7. Provisions regarding egg product stabilisation

The behaviour of microorganisms in egg products depends on the nature of the product (liquid, dried, concentrated, with salt or sugar), its initial level of contamination, the type of microorganism involved and the temperature. Whole egg and egg yolk can be rapidly highly contaminated when the refrigeration temperature is not maintained. Considering egg white, this egg product is usually bacteriostatic and can even be bactericidal under specific conditions.

Pasteurisation

The European heat treatments usually applied are temperatures of 65 to 68 °C for 5 to 6 minutes for whole egg and egg yolk. The treatments are milder for egg white (55–57 °C for 2–5 minutes) (see Baron and Jan, 2011)), due to the higher heat sensitivity of egg white proteins. These treatments are adequate to reduce vegetative flora by at least 6 logs in whole eggs or egg yolk (Baron et al., 2010). However, they are ineffective for heat-resistant flora, including spore-forming bacteria. These organisms are, however, unlikely to be involved in food-borne outbreaks due to the consumption of egg products (see Section 3.2) but may be involved in spoilage events, leading to financial losses for the egg sector.

Many experimental studies on heat resistance have been carried out on *S. Enteritidis* because of the health and food safety concerns in the egg and egg product sector, and, more recently, on *Listeria monocytogenes*, as it is known to exhibit greater thermal resistance than *S. Enteritidis* (Palumbo et al., 1995; Muriana et al., 1996; Palumbo et al., 1996; Schuman and Sheldon, 2003; Li et al., 2005; Monfort et al., 2012). It remains difficult to compare the studies, because heat resistance depends on, among other factors, the strain, the culture conditions, the inoculation size and the equipment. For the most frequent *Salmonella* serotypes, i.e. *S. Typhimurium* and *S. Enteritidis*, the D-values described in the literature range between 0.36 and 3.06 min at 60 °C for whole egg, between 0.27 and 0.35 min at 61.1 °C for egg yolk, and from 1.08 to 3.6 min at 57 °C for egg white (Humphrey et al., 1990; Shah et al., 1991; Denis et al., 1995; Palumbo et al., 1995, 1996; Doyle and Mazzotta, 2000). The D-values are higher when particularly heat resistant serotypes are studied, such as *S. Senftenberg* (Anellis et al., 1954; Mañas et al., 2003). For *Listeria*, the D-values described in the literature are in the range of 1.3 to 2.1 min at 60 °C for whole egg, 0.35 to 1.28 min at 63 °C for egg yolk and 4.35 to 20.9 min at 57 °C for egg white (Foegeding and Stanley, 1990; Bartlett and Hawke, 1995; Muriana et al., 1996; Knight et al., 1999; Michalski et al., 2000; Doyle et al., 2001).

From these data and from the z-values provided by these authors, the European heat treatments as described above for whole egg and egg yolk allow the reduction of both genera to a safe level, as has also been recently highlighted in the USA (Jin et al., 2008; Jordan et al., 2011) for this type of egg products pasteurised under the current Food Safety and Inspection Service (FSIS) standards (USDA-FSIS, 2005). However, these treatments may be critical in egg white for both genera, as well as in salted and sugared egg products (Denis et al., 1995; Palumbo et al., 1995, 1996; Knight et al., 1999; Michalski et al., 2000; Froning et al., 2002; Gurtler et al., 2013). The comment on egg white should be modulated by the fact that it is not a favourable environment for microbial development, provided that its separation from egg yolk has been carried out properly.

There are only few studies in the literature presenting data on putative survival of *Salmonella* or *Listeria* after pasteurisation. It appears clear from these few studies that this phenomenon is unlikely if stringent conditions of processing are respected and recontamination strictly controlled (McQuestin et al., 2010). Chai et al. (2012) collected data on pasteurised liquid egg products for the period 2000–2009 in the USA; only 9 samples of 15 661 tested were found to be positive, corresponding to 0.06 %. A survey of *Salmonella* in pasteurised eggs was conducted in Denmark in 2004 (Anonymous, 2006). While high levels of *Salmonella* were observed in the 294 raw egg samples analysed, only 4 of the pasteurised samples were positive. These products were salted and originated from the same company. The corresponding raw samples were contaminated at high levels (over 5 log CFU/mL). The combination of the presence of salt, the long-term storage and the relatively high temperature of cooling prior to pasteurisation was the likely explanations for the high level of contamination found. The pasteurisation conditions used by this producer were also found to be insufficient to eliminate this high bacterial load. Immediate remedial measures were imposed, including correction of time and temperature of storage and adjustment of the pasteurisation procedures. In a French study (Rivoal et al., 2009) 11 pasteurised samples out of 288 analysed were shown to be contaminated by *S. Enteritidis*. Nevertheless, the pasteurisation conditions were experimentally designed for this trial and several of them were not representative of those used under real pasteurisation conditions in France.

These studies clearly highlight the need to strictly adjust the process of pasteurisation to provide safe products, whatever the original microbial quality of the eggs, including eggs produced by *Salmonella* infected flocks and imported eggs from unknown status, as described in the Danish industry (Anonymous, 2006).

As far as the spoilage micro-organisms are concerned, the industrial processes used to produce heat treated liquid egg products are not completely effective in eliminating heat-resistant bacterial contamination. In addition to potentially leading to cross-contamination at the egg breaking step, several of these spoilage bacteria may also be further selected by the stabilisation processes. This is particularly the case for the psychrotrophic and heat-resistant species, which are potentially selected by the pasteurisation and refrigeration practices carried out in this sector (Baron et al., 2007; Pina-Pérez et al., 2009; Jan et al., 2011; Techer et al., 2014).

Other stabilisation processes

In addition to pasteurisation, there are other practices that reduce microbial development by reducing the water activity (a_w) value of the pasteurised liquid egg products, i.e. concentration, drying, freezing, sugaring and/or salting.

Drying

The concentration of whole egg or egg white, prior to drying or intended to reduce transport costs, is usually carried out by ultra filtration or reverse osmosis. It only slightly reduces the water activity (final a_w values around 0.9) and, therefore, has little influence on bacterial growth. Drying produces a powder with a_w values of 0.2 to 0.3, inhibiting any bacterial growth. This step is mainly carried out in horizontal towers, where the product is spray-atomised. The egg product powders should be stored at controlled temperatures in order to maintain their quality for long storage periods. Most dried egg products have a shelf life of up to 12 months.

Egg white is, in most cases, twice concentrated by ultra filtration prior to drying. The prior removal of free glucose avoids non-enzymatic browning due to Maillard's reaction accompanying drying. Glucose removal is mainly carried out by fermentation, involving the genera *Lactobacillus* and *Streptococcus* or the yeast *Saccharomyces cerevisiae*. The use of yeast allows rapid fermentation (two to four hours) and is easy to implement. However, it may lead to the development of unpleasant odours. After fermentation, the mixture is chilled and yeast can be removed by centrifugation. Raw egg white can also be pasteurised after drying, at temperatures of 65 °C to 85 °C for 14 to 7 days, depending on the desired functionalities of the egg white powder. This process has provided new types of ingredients, free of pathogens and exhibiting remarkable foaming, emulsifying or gelling properties.

However, attention should be paid to the possible decrease in the natural egg white defences following the reconstitution of egg white from this type of product (Baron et al., 2003).

Freezing

Freezing is another practice for stabilising egg products, as in the frozen state water is unavailable for bacteria. Once frozen, the egg products can be stored for up to 12 months.

Addition of stabilising agents

The addition of hydrophilic components such as sugar or salt also limits the water available to microorganisms. The quantities of sugar and salt can reach 50 % and 10 %, respectively, which lowers the water activity to values below 0.85.

The effect of added salt or sugar on the efficiency of pasteurisation must be carefully considered since it is generally recognised that the thermal resistance of *Salmonella* and *Listeria* increases with the addition of sugar or salt in the egg products prior to pasteurisation (Denis et al., 1995; Palumbo et al., 1995, 1996; Knight et al., 1999; Michalski et al., 2000; Froning et al., 2002; Baron et al., 2003).

Sorbic and benzoic acids are bacterial inhibitors approved for addition to liquid egg products, at concentrations of up to 5 g/kg. They may be used for their bactericidal and antifungal activities. The use of nisin is also approved, and is particularly convenient for the control of *Bacillus* species for which sorbic and benzoic acids are not effective (EFSA, 2006). These ingredients are, in most cases, added upstream of the pasteurisation step. It is important, however, to note that there is an increasing demand from consumers for foods without preservatives.

2.4.2.8. Provisions regarding the storage of liquid egg products

After pasteurisation, liquid egg products have a short shelf life due to the presence of residual non-inactivated bacteria and need to be kept refrigerated. Refrigeration reduces the growth rates of microorganisms and the ability of pathogenic bacteria to produce toxins. Significant growth of *Salmonella* is not likely to occur at temperatures below 8 °C regardless of the nature of the egg product: egg yolk (Gumudavelli et al., 2007), whole egg (Abdel Kareem and Mattar, 2001) or egg white (Clay and Board, 1991; Lock and Board, 1992; Ruzickova, 1994; Schoeni et al., 1995; Chen et al., 2005). The importance of the efficiency of the separation of egg yolk from egg white during egg breaking is crucial, as the presence of 0.5 % egg yolk in egg white is sufficient to allow *Salmonella* to grow at suitable temperatures, probably owing to the increase in iron availability (Baron, 1998). Compared with raw egg white, *Salmonella* growth is enhanced in egg white reconstituted from powder, the process of drying leading to a lowering of the egg white's natural antimicrobial properties (Baron et al., 2003).

The growth of *L. monocytogenes*, although slow, is possible at refrigeration temperatures in the three types of egg products. For egg yolk and whole egg stored at 4 °C, an increase of 2 log units was recorded in 22 days by Notermans et al. (1991) and one of 6 log units in 12 (Schuman and Sheldon, 2003) or 21 days (Foegeding and Leasor, 1990). Such differences could be ascribed to the variable presence of psychrotrophic strains in the products analysed. At the same temperature in egg white, bacteriostasis was observed by Notermans et al. (1991). Above 20 °C, growth was favoured in egg yolk (3 log units after 24 h at 22 °C) compared with whole egg (2 log units after 2–9 days at 22 °C), while egg white exhibited a bactericidal effect under the same conditions, with total destruction of an inoculum of 5 log CFU/mL being recorded in nine days at 22 °C (Notermans et al., 1991) or after 24 hours incubation at 35 °C (Wang and Shelef, 1991). This bactericidal effect was ascribed to the synergic action of alkaline pH, lysozyme, ovomucoid and ovotransferrin.

Refrigeration is therefore efficient for the control of the pathogenic flora in liquid egg products. However, it may lead to the selection of psychrotrophic microorganisms including the *Bacillus cereus* group. This microorganism is a considerable producer of enzymes and may lead to spoilage events, even at low temperatures, with significant economic consequences for the industry. Some strains are

also able to produce toxins and may lead to food poisoning issues, which, even if recognised as usually benign and rapidly resolved, may compromise the health of immunocompromised patients (Baron et al., 2007; Jan et al., 2011). It also appears crucial to control post-pasteurisation contamination by other psychrotrophic bacteria such as *Pseudomonas*. For this type of microorganism, a small increase in temperature may represent a risk. It therefore appears that maintaining the refrigeration temperature below 4 °C throughout the production chain and until egg product delivery is essential to allay safety concerns.

2.4.2.9. Provisions regarding the delivery of egg products

The type of egg product packaging varies from small size plastic units to large tankers, depending on the final destination of the product (i.e. consumer, artisan or industry). The temperature of storage and delivery of pasteurised liquid egg products should not exceed 4 °C. The shelf life depends on the type of product and on its packaging: two or three days at 4 °C for liquid egg products conditioned in bulk packages and intended for the food industry, and several days (up to 60 days) for the small packaging (1 or 2 kg) intended for artisans, catering establishments or directly for the consumer in some countries. A shelf life of several months at ambient temperature is generally recommended for dried egg products and for those with a high sugar or salt content.

2.4.3. Industrial production of specialty egg products

Other products are manufactured from table eggs, without undergoing the processing steps described above for egg products from 'first processing'. Formulated and 'ready to use' egg products are intended for commercial food production, the catering industry, restaurants, hospitals and residential facilities.

Peeled hard boiled eggs are the main products traded by volume (Galet et al., 2010). The eggs are peeled and then packed, usually in an acidic and salted solution supplemented or not with preservatives such as sodium citrate, sorbate or sodium benzoate or under atmospheric conditions. Chilled or frozen vacuum-wrapped 'tubes' of hard-boiled eggs are also produced. However, this production is anecdotal, as only a few manufacturers have the equipment to produce this foodstuff. Scrambled eggs are generally produced with liquid whole egg with or without supplementation with milk. There are different formulations depending on the country. These products are chilled (under protective atmospheric conditions) or frozen. For the production of poached and fried eggs, table eggs are broken before cooking. Poached eggs are chilled and conditioned under modified atmosphere or in a vacuum or they are salted. Fried eggs are also chilled and delivered in trays under modified atmospheric conditions. Omelettes are produced with pasteurised whole egg products often supplemented with hydrocolloids. Ingredients are added either before or after the pasteurisation step, depending on the type of equipment. After cooking, omelettes are chilled and conditioned under modified atmospheric conditions or they are frozen. Foamed egg white is one of the few sweet cooked egg products provided by the industry. For this, pasteurised liquid egg white supplemented with sugar is whipped, placed in a tray with caramel, cooked to stabilise the foam (coagulation) and quickly refrigerated. This chilled product is mainly used for the preparation of the 'île flottante' dessert.

2.5. Consumption of eggs and egg products

Data available from the EFSA Comprehensive European Food Consumption Database on consumption of table eggs from all egg-producing species in the EU are presented in Table 1. An overview of the methodologies and protocols employed in the different national dietary surveys can be found in (Mertens et al., 2011). The consumption of table eggs is variable, with the percentage of consumers ranging from around 19 % (in Latvia) to almost 99 % (in Denmark). The quantity consumed (consumers only) ranges from 14.2 g/day to 46.6 g/day for table eggs. The largest *per capita* consumer of egg products is Denmark, followed by France and Italy (126, 86, and 75 egg equivalents in the form of egg product/person/year, respectively) (Magdelaine et al., 2013).

Table 1: Average consumption of eggs (in g/day) in the EU for the total population and adult consumers only¹⁷

Country	Survey	No of subjects	Eggs, fresh		
			Average consumption for total population (g/day)	Percentage of consumers	Average consumption for adult consumers only (g/day)
Belgium	Diet_national_2004	1 304	9.5	39.1	25.0
Czech Republic	SISP04	1 666	19.5	86.1	22.6
Denmark	Danish_Dietary_Survey	2 822	16.2	98.9	16.3
Finland	FINDIET_2007	1 575	16.1	80.4	20.0
France	INCA2	2 276	15.3	86.2	17.5
Germany	National_Nutrition_Survey_II	10 419	7.1	24.0	30.3
Hungary	National_Repr_Surv	1 074	26.4	97.2	27.1
Ireland	NSIFCS	958	11.9	84.1	14.2
Italy	INRAN_SCAI_2005_06	2 313	21.3	74.3	28.7
Latvia	EFSA_TEST	1 306	8.7	18.6	46.6
The Netherlands	DNFCS_2003	750	5.0	22.8	21.8
Spain	AESAN	410	24.4	74.1	32.9
Spain	AESAN_FIAB	981	28.5	89.2	32.0
Sweden	Riksmaten_1997_98	1 210	12.3	61.4	20.0
United Kingdom	NDNS	1 724	17.1	86.0	19.8

Concluding remarks

- The pyramidal structure of the breeding of laying hens and production of table eggs results in a very large multiplication factor from the primary breeding stage. This offers an opportunity to produce pathogen-free birds, but if a vertically transmitted pathogen such as *S. Enteritidis* does enter the upper tiers of the pyramid there can be widespread dissemination of infection.
- Very large numbers of eggs are produced and consumed, and, although the prevalence of pathogens is very low, the total numbers of human cases can be large, especially if eggs are used to produce pooled dishes that are not properly heat treated or stored.
- The industrial processes of liquid egg product manufacturing reduce the level of vegetative bacteria, including *Salmonella* and *Listeria*, to a safe level, provided that effective and hygienic processing conditions are strictly implemented.
- While dried and frozen egg products may be considered to be safe for several months of storage, provided that proper storage conditions are respected, that of liquid egg products is more critical, especially those used in highly perishable products, such as chilled egg-based desserts. Liquid egg products are also more susceptible to growth of heat resistant and psychrotrophic spoilage organisms.
- The European egg product industry mainly purchases eggs from farms or from packaging centres. To the best of our knowledge, there is little evidence of significant recycling of unsold class A table eggs from retail establishments to the egg processing industry.

Recommendations

- Attention should be paid to the quality of raw egg white, as the milder heat treatments applied to this product may be insufficient to eradicate pathogenic bacteria, especially if salt or sugar

¹⁷ Available at www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

is added before pasteurisation. However, egg white does not readily support bacterial growth, which minimizes the risk.

- Attention should be paid to spore formers, such as the *B. cereus* group, which resist the pasteurisation treatment of liquid egg products and may lead to spoilage issues or possible food poisoning.
- Upstream of the breaking step, particular attention should be paid to egg freshness and external appearance. In the case of egg storage, temperature should be strictly controlled and changes in humidity should be avoided. The heat treatment times and temperatures must be strictly controlled at the pasteurisation step, in order to eradicate the vegetative forms of pathogenic bacteria.

3. Public health relevance of microorganisms transmissible through eggs

The zoonoses reports include data on food-borne outbreaks and have been mandatory for EU MSs since 2005. Since 2007 harmonised specifications for the reporting of these outbreaks at the EU level have been in use (EFSA, 2007a). In 2010, revised reporting specifications for food-borne outbreaks were implemented and the distinction between ‘verified’ and ‘possible’ food-borne outbreaks was changed to ‘strong’ or ‘weak’ based on the strength of evidence implicating a suspect food vehicle. Food-borne outbreak investigation systems at the national level are not harmonised between MSs. Consequently, the differences in the numbers and types of reported outbreaks, as well as the causative agents, may not reflect differences in food safety between MSs, but may be more indicative of differences in the efficiency and sensitivity of the national monitoring systems for identifying and investigating food-borne outbreaks. Nevertheless, this reporting of zoonoses represents the most comprehensive set of data available for the EU.

In 2012, as in previous years, the most common single foodstuff category reported as a food vehicle was eggs and egg products, responsible for 168 (22 %) out of 763 outbreaks. The majority of these outbreaks were associated with *S. Enteritidis* (67 %). Two outbreaks were caused by bacterial toxins (one each by the *Bacillus cereus* group and staphylococcal toxins). In addition, one calicivirus outbreak was attributed to eggs and egg products - see Figure 4). When eggs or egg products are used as ingredients data are insufficiently detailed to identify the specific contribution of the egg component in these outbreaks (EFSA and ECDC, 2014).

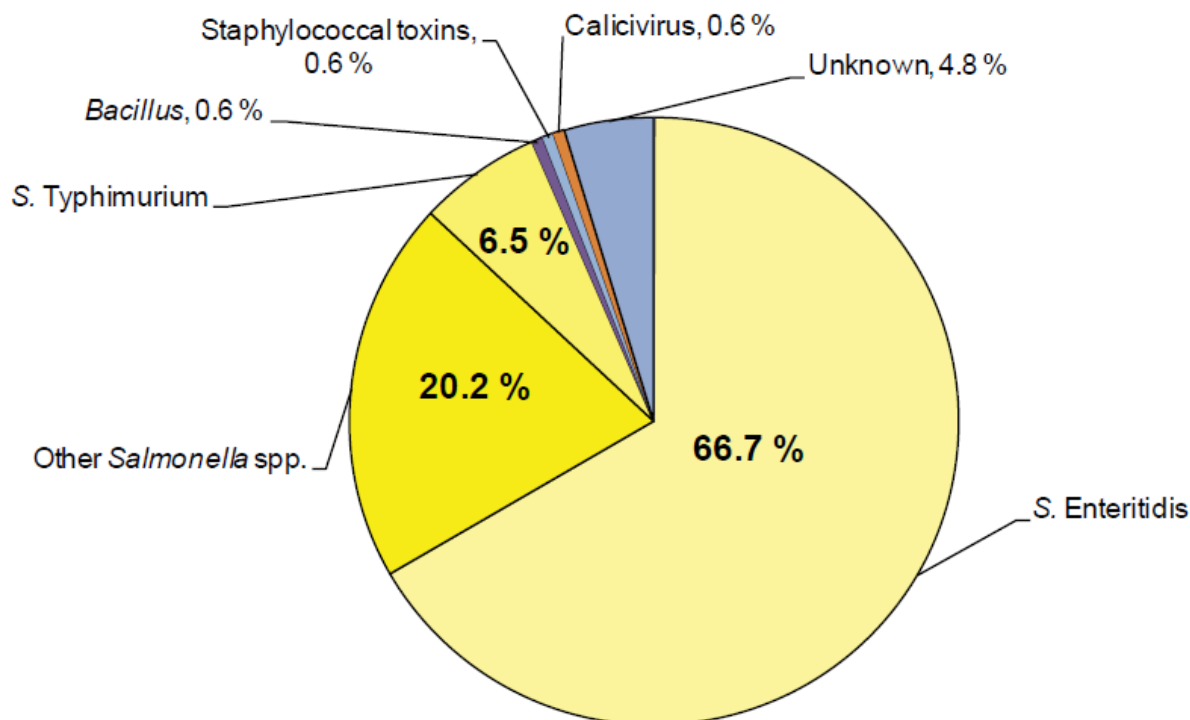


Figure 4: Distribution of strong-evidence outbreaks caused by eggs and egg products, by causative agent, in the EU, 2012. Data from 168 outbreaks are included: France (33), Germany (3), the Netherlands (1), Poland (51), Slovakia (3), Spain (74) and the United Kingdom (3) (EFSA and ECDC, 2014)

3.1. Public health significance of *Salmonella* in eggs and egg products

3.1.1. Reported and true incidence of salmonellosis in the EU

3.1.1.1. Sporadic cases

A total of 91 034 confirmed cases of human salmonellosis were reported from the 27 EU MSs through the European Surveillance System (TESSy) in 2012 (EFSA and ECDC, 2014). The EU notification rate was 22 cases per 100 000 population, ranging from fewer than 4 cases in Greece, Portugal and Romania to more than 85 cases in the Czech Republic and Slovakia. As in previous years, *S. Enteritidis* and *S. Typhimurium* were the most frequently reported serovars (41 % and 22 %, respectively, of all reported cases for which information on serovars was provided) (EFSA and ECDC, 2014).

The five-year (2008-2012) EU trend showed a statistically significant decrease in the reported incidence of human salmonellosis, although there were country-specific variations. Significant decreasing trends were observed for *S. Enteritidis* and *S. Typhimurium* (excluding monophasic *S. Typhimurium* 1,4,[5],12:i:-, which continued to increase in 2012) (EFSA and ECDC, 2013).

On average, 45 % of the confirmed salmonellosis cases were reported to be hospitalised in 2012; hospitalisation status was, however, only provided for 10 % of all confirmed cases. Fourteen MSs provided data on the outcome of their cases and, of these, 8 MSs reported a total of 61 fatal cases. This gives an EU case-fatality rate of 0.14 % among the 44 532 confirmed cases for which this information was reported (49 % of all confirmed cases) (EFSA and ECDC, 2013).

Comparison of the notification rates between countries should be made with caution because of the different degrees of underreporting between MSs. The importance of underreporting is discussed in more detail below. Comparison between years within a country is, in general, more valid.

The total number of reported cases includes sporadic, travel- and outbreak-related infections. It should be noted that the proportion of travel-related cases in 2012 was, as usual, very high, more than 70 % in the Nordic countries: Finland, Sweden and Norway (EFSA and ECDC, 2014).

3.1.1.2. Underreporting

Several Scientific Opinions from the BIOHAZ Panel related to setting of targets in poultry populations provide detailed information on underreporting of human salmonellosis (EFSA BIOHAZ Panel, 2010b, 2011, 2012). Details of the reporting system for human salmonellosis in the EU can also be found in the EU Summary Reports (EFSA and ECDC, 2010, 2011).

The true incidence at population level may be considerably greater than the reported one, albeit the level of underreporting varies strongly between MSs (de Jong and Ekdahl, 2006). ‘Multipliers’ (i.e. the ratio between true and reported cases) can be found in scientific papers referring to single countries and, for example, range from 4.7 for the United Kingdom (Tam et al., 2012), to 29.3 for the USA (Scallan et al., 2011).

Underreporting values for human salmonellosis in the different EU MSs were estimated employing updated information on the risk from Swedish travellers in the EU in 2009, as described in detail by the EFSA BIOHAZ Panel (2011). The underreporting factor at the EU level is estimated to be 57.5 (95 % confidence interval (CI) 9.0–172). For the EU-27, the estimated true incidence of salmonellosis in 2009 was estimated as 6.2 (95 % CI 1.0–19) million cases. The disease burden of salmonellosis and its sequelae is 0.23 million (95 % CI 0.05–0.6 million) disability-adjusted life-years (DALYs) per year and total annual costs were estimated at EUR 2 billion (95 % CI EUR 0.3–4 billion) (EFSA BIOHAZ Panel, 2011). It is estimated that in 2010 there were approximately 5.4 million (95 % CI 3.0–9.5 million) true cases of human salmonellosis in the EU-27, a 13 % decrease compared with 2009 (EFSA BIOHAZ Panel, 2012).

3.1.2. Attributing eggs and egg products to human salmonellosis

3.1.2.1. Outbreak analysis

From 2009 to 2012, 17 MSs and one non-MS (Switzerland) reported a total of 610 food-borne outbreaks of human salmonellosis implicating eggs or egg products. These outbreaks were supported by strong evidence and resulted in 6 339 cases, 1 554 hospitalisations and 10 deaths. *S. Enteritidis* was the predominant serovar associated with these outbreaks, accounting for 437 (72 %) of outbreaks and 76 % of human cases (4 842). Most outbreaks, 74 % (273), took place in the ‘household’ setting. Only 28 outbreaks were linked to the ‘Restaurant, Cafe, Public House/Inn, Bar, Hotel’ reporting categories¹⁸.

Many contributing factors, either alone or in combination, were reported in these outbreaks. The most common were unprocessed contaminated ingredients, inadequate heat treatment and infected food handlers.

The reporting of outbreaks by the different MSs depends very much on the resources in place for handling these incidents. Furthermore, large outbreaks or those involving unusual serovars will have a greater probability of being detected by the surveillance systems in an MS than smaller outbreaks of common serovars. The chance to verify the causative agent is also inherently associated with the incriminated food vehicle and source. If the combination of food vehicle and the causative agent is frequently linked and associated with outbreaks (e.g. *S. Enteritidis* in eggs) it may be anticipated that

¹⁸ Data on outbreak setting not available for all countries.

the chance to verify the outbreak will be greater, as the potentially implicated food vehicle will most likely be included in epidemiological and microbiological investigations.

3.1.2.2. Source attribution modelling

A variety of methods to estimate the relative contribution of food-producing animal sources to human foodborne disease have been developed, including the microbial subtyping approach, comparative exposure assessment, epidemiological analyses of sporadic cases, analysis of data from outbreak investigations, and expert elicitations (EFSA, 2008; Pires et al., 2009). To assess the public health impact of setting new targets for the reduction of *Salmonella* in fattening turkeys, source attribution modelling using the microbial subtyping approach was applied (EFSA BIOHAZ Panel, 2012). The model is referred to as the 'Turkey-Target *Salmonella* Attribution Model' (TT-SAM). Detailed information can be found in the external report provided by the contractor (Hald et al., external contractor report).

TT-SAM included 25 MSs, four animal-food sources and 23 *Salmonella* serovars. It employed (i) prevalence and serovar distribution data from the 2010 EU statutory monitoring (turkeys, broilers and laying hens) and the EU-wide baseline survey conducted in 2006–2007 for slaughter pigs (EFSA, 2008); (ii) data on incidence and serovar distribution of reported cases of human salmonellosis in 2010 provided by the European Centre for Disease Prevention and Control (ECDC); and (iii) food availability data, including amounts traded between MSs. MS-specific underreporting factors for human salmonellosis were applied (see Section 3.1.1.2).

The results of the TT-SAM model indicate that the true number of human salmonellosis cases (i.e. accounting for underreporting) in the EU in 2010 was estimated to be 5.4 million (95 % CI 3.0–9.5 million) as described above. The model estimated that 17.0, 2.6, 56.8 and 10.6 % of human salmonellosis cases could be attributed to the laying hen (eggs), turkey, pig and broiler reservoirs, respectively. The laying hen (eggs) reservoir is estimated to correspond to around 928 000 (95 % CI 443 100–1 878 000) true human cases in 2010.

The model estimated that approximately 87 % of the laying hens (eggs)-associated human salmonellosis cases were caused by *S. Enteritidis*. *S. Infantis* accounted for 3.2 % of all laying hen (eggs)-associated human salmonellosis, *S. Virchow* 2.2 % and *S. Typhimurium* 1.8 %. Other serovars accounted for less than 1 % on an individual basis. A recent review has suggested, however, that egg-borne outbreaks (and cases) caused by serovars other than *S. Enteritidis* are nevertheless quite substantial (Threlfall et al., 2014).

3.2. Hazards other than *Salmonella*

Although eggs and egg products are often incriminated in outbreaks caused by *S. Enteritidis*, they are also susceptible to contamination by other pathogens, such as *B. cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Campylobacter*.

In 2011, eggs or egg products were shown to be involved in two outbreaks due to *B. cereus* group toxins (EFSA and ECDC, 2013). However, the role of eggs or egg products in human infection is not clear. In a study performed on 50 conventional laying farms in western France, Koné and colleagues (2013) assessed the presence of *B. cereus* group bacteria after enrichment of the eggshell flora. The results showed a generally low frequency of eggshell contamination by this bacterial group in newly laid eggs. The data showed that eggshell contamination was influenced by the hygienic practices of the farmers, particularly when the farms were composed of several buildings and biosecurity practices were poorly implemented. Although the prevalence of these bacteria was described as low on the surface of table eggs, they may be selected by the industrial processes of egg product stabilisation (pasteurisation and chilling) and by their development in the form of biofilms on the stainless steel processing equipment (Andersson et al., 1995). They may reduce the microbiological quality of the egg products (see Section 11.4) by leading to spoilage, as they are known to produce spoilage enzymes (Baron et al., 2007; Jan et al., 2011; Techer et al., 2014). The control of their presence appears to be

less relevant in shell egg production than in the industrial environment of egg product manufacturing. The involvement of eggs or egg products in the *B. cereus* group related problems may be ascribed to the fact that eggs and egg products are intermediate products used for the processing of many industrial foods and are also widely used in institutional and commercial catering. These locations accounted for 87 % (49 % in schools) of French household food-borne diseases related to this group between 2006 and 2010 (Cadel Six et al., 2012). According to EFSA and ECDC (2014), in strong-evidence *B. cereus* group outbreaks, mixed food was the most commonly implicated food vehicle (28.9 % of outbreaks). The second most frequently reported implicated single food vehicle was fish and fish products (13.2 % of outbreaks) followed by cereal products (10.5 % of outbreaks). Foodborne intoxications due to this bacterial group are recognised as generally mild and rapidly resolved and clinical diseases are mainly associated with high levels of exposure. However, the *B. cereus* group has also been described as being responsible for several episodes of fatal illnesses (Lund et al., 2000; Dierick et al., 2005; Bottone, 2012). The possibility of underreporting should be considered due to the possible confusion between the symptoms caused by the *B. cereus* group toxins and those caused by *Clostridium perfringens* and *S. aureus* toxins, which may lead to misdiagnosis (Cadel Six et al., 2012).

Staphylococci have the potential to cause spoilage and enter the food chain, resulting in food-borne disease. Increasing attention has been given to the role of poultry and poultry products, including eggs, as a potential source of infections in humans induced by antibiotic-resistant *Staphylococcus* strains (Manie et al., 1998; Abulreesh and Organji, 2011). Most of these have the ability to produce one or more enterotoxins, which in many cases are the cause of serious food poisoning in humans (Hatakka et al., 2000; Tamarapu et al., 2001). Bacterial contamination of egg contents could result from the penetration of the shell by bacteria deposited on the surface of the egg during passage through the cloaca or after it has been laid (Bahrouz and Al-Jaff, 2005). Wieneke et al. (1993) reported that in Great Britain, between 1969 and 1990, 3.5 % of cases of staphylococcal food poisoning were caused by eating eggs contaminated by *S. aureus*. In France, 11 % of cases of food poisoning in 1999-2000 resulted from eating eggs and egg products contaminated with staphylococci (Haeghebaert et al., 2002). In localised outbreaks of food poisoning and food-borne infection in Poland, 2.8 % of cases were linked to eggs contaminated with *S. aureus*. In 2001 this figure reached 6.9 % (Przybylska, 2002, 2003), and in 2009 it was shown that 25 % of food poisoning cases induced by *S. aureus* in humans were caused by consumption of table eggs (Baumann-Popczyk and Sadkowska-Todys, 2011). In a study performed in 2012, Pyzik and Marek (2012) analysed 70 table quails' eggs purchased at retail in Lublin; 45 strains of the genus *Staphylococcus* were isolated from the shell and the content, and 7 out of 45 were *S. aureus*. Pyzik and Marek (2013), in a study on 90 table chicken eggs, isolated 105 bacterial strains identified as *Staphylococcus*, of which 18 (17.1 %) were *S. aureus*; 55.5 % of the strains were isolated from the shell, 27.8 % from the yolks and 16.7 % from the albumen. In addition, Stepień-Pyśniak and colleagues, in a study carried out in (2009), found staphylococci in 45.7 % of the analysed eggs. In the egg yolk, the dominant species was *S. aureus*, but this bacterium was isolated less frequently from the eggshell surfaces than from yolks.

Listeria monocytogenes is not frequently studied in poultry flocks, and poultry products are rarely involved in cases of listeriosis. A few studies have investigated the prevalence of *L. monocytogenes* on eggshells; they all found that samples tested negative for this pathogen (Adesiyun et al., 2005; Busani et al., 2005). Nevertheless, an investigation carried out in poultry flocks in France revealed that *L. monocytogenes* was present in 30 % of caged hen flocks (Chemaly et al., 2008). The most frequently detected serotype in poultry was 1/2a (84 %), which is the second most common cause of human listeriosis, after serogroup 4 (Goulet et al., 2004). *L. monocytogenes* may present a risk to egg and egg product consumers because of its ability to contaminate eggshells and occasionally transfer to egg products (Protais et al., 2007; Rivoal et al., 2010). Similar pulsed-field gel electrophoresis pulsotypes have been found in strains isolated from laying hen flocks and egg products, suggesting the causal role of the primary production in introducing *L. monocytogenes* into the processing plants (Rivoal et al., 2013).

Campylobacter contamination is more often associated with poultry meat and broiler flocks, in which its prevalence can reach 100 % (EFSA, 2010). This pathogen can also be found in laying hen farms

(Doyle, 1984; Rasschaert et al., 2007; Sulonen et al., 2007; Schwaiger et al., 2008; Cox et al., 2009; Messelhäuser et al., 2011). The prevalence of *Campylobacter* in laying hen farms varies among studies and countries from 35 % (Rasschaert et al., 2007) to 84 % (Sulonen et al., 2007). *Campylobacter* is most frequently isolated from caeca but is also able to colonize other organs and tissues such as the ovarian follicles, the reproductive tract, the spleen and the liver–gallbladder (Cox et al., 2009). Although the ability of *Campylobacter* to colonize reproductive tracts has been reported, there is no evidence for vertical transmission of the pathogen (Sahin et al., 2003; Callicott et al., 2006; Fonseca et al., 2006). Eggshell contamination by *Campylobacter* has not been widely investigated. Some studies have reported that the presence of *Campylobacter* on eggshells is uncommon despite flock infection (Doyle, 1984; Shane et al., 1986; Jones and Musgrove, 2007; Schwaiger et al., 2008; Widdicombe et al., 2009). Shane et al. (1986) demonstrated that *C. jejuni* cannot survive more than 16 hours in a dry environment. A study performed in Trinidad found that 1.1 % of sampled table eggs were contaminated by *Campylobacter* (Adesiyun et al., 2005), and another one in Germany reported 4.1 % of eggshells sampled contaminated by this bacterium, which was nearly four times higher than the rate of contamination for *Salmonella* (Messelhäuser et al., 2011). In the EFSA Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU (EFSA BIOHAZ Panel, 2010c), 1.3 % of *Campylobacter* outbreaks were attributed to eggs, through source attribution based on outbreak investigation data. However, Pires et al. (2010) questioned the reliability of using outbreak data for source attribution for *Campylobacter*, because the relative contribution of sources to sporadic and outbreak associated disease appears to differ, which could bias estimates based only on outbreak data.

Very few studies have reported the occurrence of *Campylobacter* and *L. monocytogenes* in egg products (Izat and Gardner, 1988; Busani et al., 2005; Protais et al., 2007; Ohkochi et al., 2009; Rivoal et al., 2010; Sato and Sashihara, 2010). The reported incidence of *Campylobacter* and *L. monocytogenes* varied between countries and studies. In France, the reported prevalence of *Campylobacter* and *L. monocytogenes* in raw egg product samples was 4 % and 17 %, respectively (Protais et al., 2007; Rivoal et al., 2010). In Japan, *Campylobacter* was isolated from 27.9 % of raw liquid whole eggs (Sato and Sashihara, 2010), while *L. monocytogenes* was detected in 8 % and 55 % of the samples depending on the egg-breaking facility (Ohkochi et al., 2009). The authors found that pasteurization eliminates *Campylobacter* contamination, confirming that the pathogen is heat sensitive in these products. Meanwhile, *L. monocytogenes* could still be detected after pasteurization (Protais et al., 2007; Ohkochi et al., 2009; Sato and Sashihara, 2010). *L. monocytogenes* contamination appears to be season-dependent and it is higher during summer and winter than during autumn with an apparent dominance of the serovar 1/2a (Rivoal et al., 2010). The contamination of the egg products is, in most cases, the result of cross-contaminations during the cracking of contaminated eggshells.

There have been no reported outbreaks due to pathogenic *Escherichia coli* linked to eggshells or egg contents, but colonization of the chicken intestinal tract with *E. coli* O157:H7 is possible (Berry et al., 1985; Schoeni and Doyle, 1994; Kabir, 2010). Faecal material has the potential to promote the growth of *E. coli* O157:H7 and *S. aureus* on the eggshell. It has been demonstrated that bacterial invasion of pores on shells occurred at a high incidence when eggshells with an amorphous cuticle were moistened and challenged with a bacterial culture. However, a low incidence of penetration was recorded when a vesicular dry cuticle was challenged with bacteria in the same manner. Egg cooling (10 °C) was inefficient in controlling the penetration of *E. coli* O157:H7 when it was present at high levels of contamination (5 log₁₀ CFU/g faeces). This high contamination overcame the shell barrier within one or two weeks even at a low storage temperature of 10 °C, but it required a long storage time of at least one month to be recovered from albumen or yolk. On the other hand, in low microbial load conditions, organisms may have no invasion potential or do not survive under low storage temperatures. The risk of penetration increases with the increase in inoculum levels present on the outer shell and thus may lead to more extensive shell structural changes (Al-Natour et al., 2012).

Very low contamination (less than 1 log₁₀ CFU/mL) of extended-spectrum beta-lactamase-producing *E. coli* (ESBLEC) was found by direct plate count on the shell of eggs purchased in different

supermarkets and grocery shops in Spain. No ESBLEC was found after 24 and 48 hours incubation in chicken eggshells (Egea et al., 2011).

Chlamydia psittaci is an obligated intracellular gram-negative bacterium; it is a zoonotic agent causing psittacosis or parrot fever in humans. In a study performed by Dickx and Vanrompay (2011), *C. psittaci* was not detected on either eggshells or eggshell membranes.

Arcobacter spp., members of the Campylobacteraceae, have recently been considered to play a role as potential zoonotic microorganisms (Ho et al., 2006). *Arcobacter butzleri*, *A. cryaerophilus*, and *A. skirrowii* have been detected in diseased, as well as in healthy, humans and animals. In humans, the bacteria were isolated from several cases of bacteraemia (On et al., 1995; Hsueh et al., 1997; Yan et al., 2000; Woo et al., 2001; Lau et al., 2002; Wybo et al., 2004) and were found both in stool specimens of healthy and diarrheal humans (Vandenberg et al., 2004; Houf and Stephan, 2007; Samie et al., 2007). No evidence was found (Lipman et al., 2008) for transmission of *Arcobacter* spp. from breeding hens to eggs.

C. burnetii was detected (Tatsumi et al., 2006) in market chicken eggs and mayonnaise in several countries, including Japan. It was detected by nested polymerase chain reaction (PCR) assay in the 4.2 % of eggs, but there are no records of Q fever outbreaks related to the consumption of eggs.

Concluding remarks

- *Salmonella* spp. accounted for more than 85 % (*S. Enteritidis* for more than 65 %) of strong evidence outbreaks caused by eggs and egg products in the EU, according to official data from 2012.
- Various source attribution models have been developed to estimate the relative contribution of food-animal species to human salmonellosis; among these, the TT-SAM model indicated the true number of human salmonellosis cases in the EU in 2010 was estimated to be 5.4 million, with 17 %, 2.6 %, 57 % and 11 % of cases attributed to the laying hen, turkey, pig and broiler reservoirs, respectively.
- Among pathogens other than *Salmonella* that can cause egg-borne diseases, there are a small number of outbreaks due to *S. aureus* and *B. cereus* group toxins in the EU. Other microorganisms, such as *L. monocytogenes*, can be found on or in eggs, but eggs are not reported as being a cause of outbreaks.

4. Eggs from other species

Production of table eggs from other species, such as ducks, quail, geese, turkeys, ostriches and seagulls, varies between countries and typically eggs are sold as a small-scale alternative, niche or luxury commodity. Improved standards, including egg date stamping and vaccination of birds against *Salmonella* are, however, being increasingly used by larger producers. Birds are typically housed on straw with floor-level nest boxes and open water troughs, which can lead to high litter moisture levels and faecal soiling of eggs. Eggs are normally collected manually several times a day and washing and/or bleached. This may remove the cuticle of the egg and theoretically make it more susceptible to trans-shell penetration, but chlorine bleaching appears to be highly successful in reduction of bacteria on shell surfaces, thus probably resulting in a lower overall risk.

Duck egg production is typically less structured than the chicken egg industry (Huang and Lin, 2011), often with home breeding of birds, lower biosecurity standards and multiple age production, which limit the opportunities for all-in-all-out management. Moulting of birds is done frequently, or old birds from large producers may be sold on to smaller producers after a year of lay. In most countries there is no formal *Salmonella* control programme for ducks.

Production of goose and turkey eggs is typically small scale, and eggs may be a by-product of small meat bird breeding flocks, although the public health risk associated with consumption of fertile eggs

is increased (Kottwitz et al., 2013). Flocks are normally floor housed on straw bedding in semi-open or free-range buildings on mixed livestock farms. Eggs are collected manually. Quail egg production may be more intensive, with birds housed either in floor pen systems or cages (Shanaway, 1994).

In most countries there is no formal *Salmonella* control programme for ducks and infection may be common (Adzitey et al., 2013; Cha et al., 2013). As duck eggs are typically dirtier than hens' eggs when collected, there is a strong chance of faecal contamination of shells by other commonly occurring intestinal organisms, including *Campylobacter* and *Listeria* (Adzitey et al., 2012). Internal contamination of eggs appears to be unusual (Nor Faiza et al., 2013), although this may depend on the numbers of eggs examined and hygienic conditions (Saitanu et al., 1994; Rezk and Saleh, 2008; Nor Faiza et al., 2013).

Outbreaks related to the consumption of duck eggs contaminated with *S. Typhimurium* definitive phage type (DT) 8 have been reported in the United Kingdom, Northern Ireland and the Republic of Ireland (Noble et al., 2012; Garvey et al., 2013). The link between human illness and duck eggs was supported by descriptive epidemiology and microbial evidence (Garvey et al., 2013). The outbreaks were related (although definitive evidence could not be provided) to a breeding company persistently infected with *S. Typhimurium* DT8, which was supplying infected day-old ducklings (Noble et al., 2012). *S. Enteritidis* cases have also been reported (Nastasi et al., 1998), but the duck-associated phage type (PT) 9b is very rarely reported in human cases.

Data on quail egg production are scarce and it is difficult to estimate the importance of these eggs across the EU. Quail egg production may be more intensive, with birds housed either in floor pen systems or cages (Shanaway, 1994). Young quail start to lay at around 6-12 weeks old and the eggs are typically collected by hand. Birds may have access to an outside veranda area and are often kept in groups of several thousand on shavings. Eggs are laid on the floor, but the birds tend to congregate in one area for egg laying. Laying birds are placed for 35-40 weeks, during which time each bird is expected to lay around 150 eggs. There is little information on the zoonotic risk associated with quail eggs, but *S. Enteritidis* infection has been reported from some countries (Porter, 1998; Javan et al., 2012; Katayama et al., 2013). These data, like most of that relating to duck eggs, originate from Middle East or Far East countries, and are difficult to extrapolate to the current EU situation. Studies have shown that the potential for temperature-related growth, enhancement of contamination by temperature fluctuations and contamination of pooled egg dishes and the kitchen environment are similar for quails' and hen's eggs (Aikawa et al., 2002). A low potential for trans-shell contamination and rapid die-off of shell contamination over time has also been observed (Katayama et al., 2013). *L. monocytogenes* can also be found on quails' eggs, leading to contamination of liquid egg (Erdogru, 2004).

There is little information on zoonotic pathogens in laying geese flocks, but similar *Salmonella* infections to those found in ducks, as well as egg contamination, may occur (Yu et al., 2008; Jahantigh, 2013). The same applies to turkeys, but *S. Enteritidis* is very rare in turkey flocks and *Salmonella* from turkeys is considered to contribute little to human infection (EFSA BIOHAZ Panel, 2012; EFSA and ECDC, 2014). Ostriches are normally housed outdoors, and *Salmonella* has been occasionally reported in flocks (de Freitas Neto et al., 2009; Akbarmehr, 2010).

It is apparent that the zoonotic risks associated with different species of poultry involved in small scale egg production are similar (Dale and Brown, 2013) and the risk in relation to eggs is highly influenced by the cleanliness of housing conditions, moisture levels and the effectiveness or otherwise of interventions such as egg washing.

Concluding remarks

- There is little information at the EU level on the production and consumption of eggs other than those from hens.

- There are no formal *Salmonella* control programmes applied in the EU in species other than *Gallus gallus*, and infection may be common in some species, such as ducks
- The role of *S. Enteritidis* in these species is not as prominent as in *Gallus gallus*, while *S. Typhimurium* appears relevant in ducks and has been reported to cause egg-borne outbreaks.
- There are insufficient data to assess the effect of increasing storage time of eggs from species other than *Gallus gallus* on the public health risk derived from consumption of eggs.

Recommendations

- In order to assess the public health risk arising from the consumption of eggs from species other than *Gallus gallus*, collection of data on the production, use and consumption of eggs from these species in the EU is recommended.
- In order to assess the effect of increasing storage time of eggs from species other than *Gallus gallus* on the public health risk derived from consumption of eggs further study is recommended to obtain more data on the structure of the eggs from these species, e.g. shell permeability, inhibitory properties of the albumen, dynamics of membrane breakdown, multiplication of *Salmonella*; as well as the frequency of shell and internal egg contamination by relevant hazards.

5. EU-regulated monitoring and control measures for *Salmonella* in laying hen flocks

5.1. Implementation of control programmes and monitoring results since 2008

Directive 2003/99¹⁹ provides for the monitoring of zoonoses in animal populations in Europe.

The first specifications for criteria for *Salmonella* monitoring were laid down in Regulation 2160/2003²⁰, which in annex II lists the minimum requirements that food business operators have to achieve in terms of testing for the control of *Salmonella* in regulated animal species and production sectors. As far as laying flocks of *Gallus gallus* are concerned, the Regulation requires all *Salmonella* strains with public health significance to be monitored, during both the rearing and the laying phase.

Before setting the targets for the reduction of the prevalence of certain *Salmonella* serotypes in laying flocks, a baseline study was organised in all EU MSs in 2004-2005. This revealed a much higher prevalence of infected flocks than had previously been identified by routine monitoring at national level (EFSA, 2007a, 2010). This means that since 2008 most MSs have easily met the original prevalence targets that were set (Figure 5).

With Commission Regulation (EU) No 517/2011²¹, which amended Regulation 1168/2006²², definitive final target of 2 % for the reduction of the prevalence of serotypes Enteritidis and Typhimurium in laying flocks of *Gallus gallus* were set, and the testing scheme necessary to verify achievement of the target described.

¹⁹ Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31-40.

²⁰ Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents. OJ L 325, 12.12.2003, p. 1-15.

²¹ Regulation (EC) No 517/2011 of 25 May 2011, implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010. OJ L 138, 26.5.2011, p. 45-51.

²² Commission Regulation (EC) No. 1168/2006 implementing Regulation (EC) No. 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No. 1003/2005. OJ L 211, 1.8.2006, p. 4-8.

According to Regulation (EC) No 1177/2006²³ vaccination programmes against *S. Enteritidis* reducing the shedding and contamination of eggs shall be applied at least during rearing to all laying hens in MSs if they have not demonstrated a prevalence below 10 % based on the results of the baseline study or based on the monitoring to follow up the EU target.

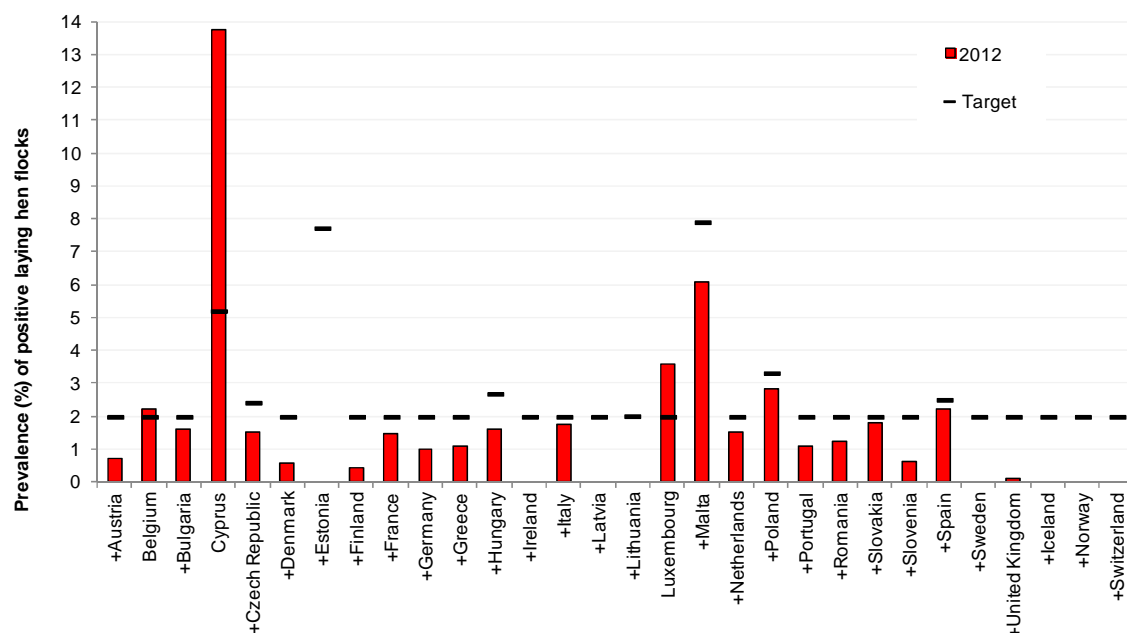


Figure 5: Prevalence of *S. Enteritidis* and/or *S. Typhimurium*-positive laying hen flocks of *Gallus gallus* during the production period and targets for Member States, Iceland, Norway and Switzerland, 2012 (from EFSA and ECDC (2014))

5.2. *Salmonella* prevalence and serovar distribution among isolates in table eggs

In 2012, 16 MSs reported data from investigations in table eggs with 25 or more samples. The prevalence of *Salmonella* positive samples for both individual samples (11 523 units tested) and batches (7 320 batches) was 0.1 % (EFSA and ECDC, 2014). Information on serovar distribution in table eggs and egg products from *Gallus gallus* over the period 2004–2012 is presented in Table 2. *S. Enteritidis* was the most prevalent serovar, followed by *S. Typhimurium* and *S. Infantis*. Monophasic *S. Typhimurium* was detected in poultry and meat from broilers, but it has never been reported among the *Salmonella* isolates from eggs over this period.

²³ Commission Regulation (EC) No. 1177/2006 implementing Regulation (EC) No. 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry. OJ L 212, 2.8.2006, p. 3-5.

Table 2: *Salmonella* serovars from eggs or egg products reported in the context of the Zoonoses Directive 2003/99/EC

Serovar	2004-2012	
	N	%
<i>S. Enteritidis</i>	516	82.56
<i>S. Typhimurium</i>	54	8.64
<i>S. Infantis</i>	18	2.88
<i>S. Montevideo</i>	11	1.76
<i>S. Braenderup</i>	6	0.96
<i>S. Bareilly</i>	4	0.64
<i>S. Kottbus</i>	2	0.32
<i>S. Mbandaka</i>	2	0.32
<i>S. Duisburg</i>	1	0.16
<i>S. Gallinarum</i>	1	0.16
<i>S. Indiana</i>	1	0.16
<i>S. London</i>	1	0.16
<i>S. Sandiego</i>	1	0.16
<i>S. Kentucky</i>	1	0.16
<i>S. Livingstone</i>	1	0.16
<i>S. Napoli</i>	1	0.16
<i>S. Wentworth</i>	1	0.16
<i>S. Bredeney</i>	1	0.16
<i>Salmonella</i> spp.	2	0.32
Total	625	

It should be noted that these data are to be regarded only as indicative, as the reported serovars often originate from different sampling schemes and as there are differences between the MSs in the way in which reports are made and the numbers of serovars reported.

There are limitations in the ability to detect *Salmonella* in eggs, such as the number tested and sampling methods (Carrique-Mas and Davies, 2008). *Salmonella*-positive flocks of laying hens produce a small number of contaminated eggs (Humphrey et al., 1989a; Davies and Breslin, 2003; Carrique-Mas and Davies, 2008; Arnold et al., 2014a). With a low prevalence of individual egg contamination, large numbers of eggs have to be tested to obtain an accurate measure of egg contamination rates (Carrique-Mas and Davies, 2008). Furthermore, the production of contaminated eggs by *S. Enteritidis*-infected hens has been found to be clustered but intermittent in a study involving naturally infected hens (Humphrey et al., 1989a).

Field studies have found a correlation between the number of positive environmental samples and the proportion of eggs positive in a flock (Kinde et al., 1996; Davies and Breslin, 2004; Chemaly et al., 2009; Renu and Tripathi, 2011), suggesting that prevalence of infection and on-farm hygiene are directly related to the number of contaminated eggs produced (Arnold et al., 2014b). Eggshell contamination occurs more frequently than the contamination of the egg contents (Wilson et al., 1998; Davies and Breslin, 2004; Little et al., 2007b; Little et al., 2008). There is a non-linear (quadratic) relationship between infection prevalence in laying hens and the rate of eggshell contamination but a linear relationship between the rate of contamination of egg contents and the prevalence of infected laying hens in a flock, with eggshell contamination occurring at a much higher rate than that of egg contents (Arnold et al., 2014a). Eggshell-contaminated eggs pose a risk of cross-contamination in the kitchen, but the risks associated with this contamination have yet to be assessed (Luber, 2009).

Salmonella serovars differ in their ability to cause contamination on the eggshell or the egg contents (Raspoet et al., 2013). In a recent study, a significant difference was found in the rate of egg contamination between serovars, with *S. Enteritidis* causing a higher rate of contamination of egg contents and a lower rate of contamination of eggshells per infected hen (0.32 % and 0.34 %, respectively).

respectively) than *S. Typhimurium* (0.23 % and 0.94 % respectively) and to non-*S. Enteritidis*, non-*S. Typhimurium* serovars (0.23 % and 2.5 % respectively) (Arnold et al., 2014a).

In Europe, *S. Enteritidis* is the serovar most commonly associated with egg contamination in most of the egg and laying hen surveys carried out (Martelli and Davies, 2012; EFSA and ECDC, 2013). Food-borne outbreaks related to the consumption of *S. Enteritidis*-contaminated eggs are widely reported in Europe and globally (Gormley et al., 2012; Liu et al., 2012; Zielicka-Hardy et al., 2012; Davies et al., 2013; Zenner et al., 2013; Harker et al., 2014).

S. Typhimurium is associated with eggs to a lesser extent than *S. Enteritidis* in Europe (EFSA and ECDC, 2013), but is the serovar mostly associated with laying hens and eggs in a small number of other geographical areas worldwide (Jamshidi et al., 2010; Singh et al., 2010; Chousalkar and Roberts, 2012). In France, for example, *S. Typhimurium* 4,5,12:- (aphasic, non-motile) has been recently linked to a foodborne outbreak related to the consumption of tiramisu (Le Hello et al., 2012).

Other *Salmonella* serovars (e.g. *S. Senftenberg*, *S. Livingstone*, *S. Infantis*) are occasionally isolated from eggs, mainly from eggshells, but also rarely from egg contents (Martelli and Davies, 2012). *S. Infantis* has been isolated from eggs in several surveys carried out in Europe ((de Louvois, 1993; Little et al., 2007a; Murchie et al., 2007; Wilson, 2007; Chemaly et al., 2009; Martelli and Davies, 2012). *S. Infantis* is also associated with egg and human illness in geographical areas outside Europe, such as Japan and New Zealand (Lapuz et al., 2008; Wilson, 2007).

5.3. Impact of the *Salmonella* control programmes

To evaluate the impact of the *Salmonella* control programmes on public health, the incidence of human salmonellosis cases caused by *S. Enteritidis*, the numbers of *Salmonella* food-borne outbreaks caused by eggs and egg products and the prevalence of *S. Enteritidis* in laying hen flocks were examined by EFSA and ECDC (2013). At the EU level, the proportion of *S. Enteritidis*-infected laying hen flocks during the production period decreased steadily from 3.9 % in 2007 (19 reporting MSs) to 1.3 % in 2011 (27 reporting MSs). During the same period the proportion of *Salmonella* spp. positive table eggs decreased from 0.8 % in 2007 (16 reporting MSs) to 0.1 % in 2011 (13 reporting MSs) (Figure 6). In the same period, a 60.5 % drop in the notification rate of human *S. Enteritidis* cases per 100 000 population was observed (from 21.0 to 8.3). A corresponding 42.3 % reduction in the number of *Salmonella* spp. food-borne outbreaks caused by eggs and egg products was reported in the EU from 2007 to 2011 (a decrease from 248 to 143 outbreaks) (Figure 6). The decline in the occurrence of *S. Enteritidis* continued in 2011 both in laying hens and their eggs and in human cases.

It should be noted that the *Salmonella* control programmes now in place in MSs are intended to have an impact on the whole food chain from farm to fork and that a reduction in *Salmonella* at the farm level is expected to reduce the risk of salmonellosis in humans. In addition, other control measures along the food chain, during slaughter, processing, distribution, retail and food preparation, are also important in reducing the risk. The results above indicate that the reduction of *S. Enteritidis* in laying hen flocks and of *Salmonella* spp. in table eggs is likely to have contributed to the decline of *S. Enteritidis* cases in humans.

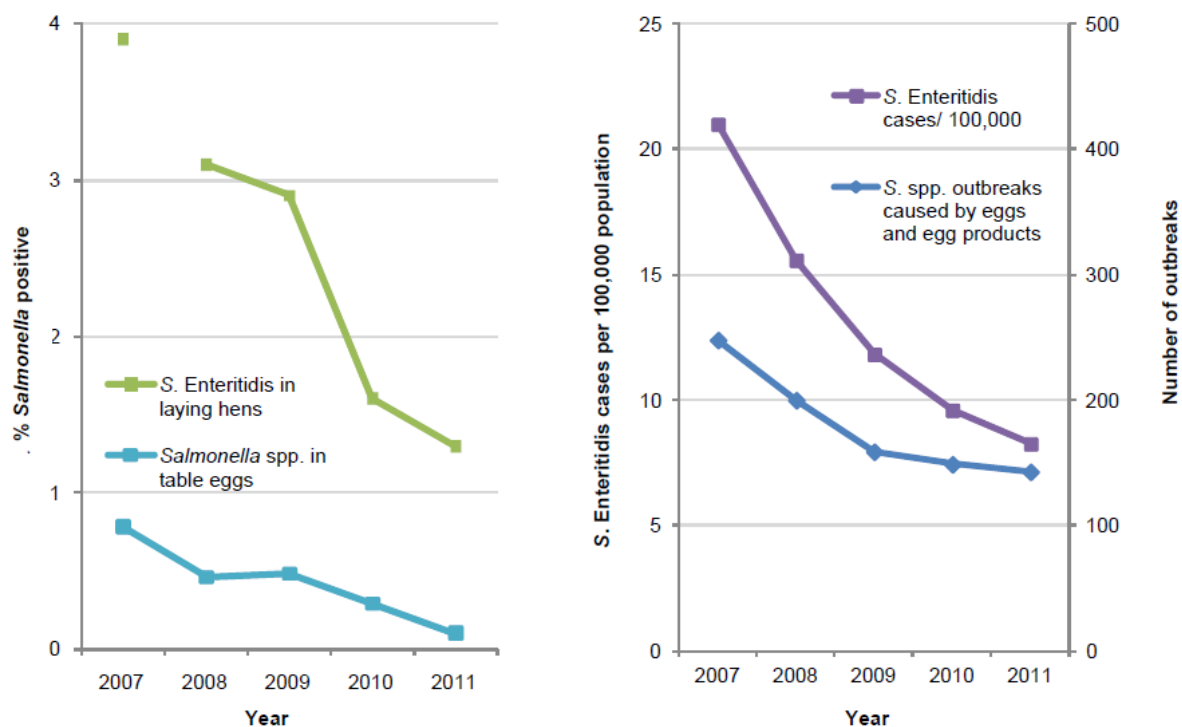


Figure 6: *Salmonella* in human cases, eggs and laying hens and the number of *Salmonella* outbreaks caused by eggs within the EU, 2007–2011 (EFSA and ECDC, 2013). Data for table eggs are presented only for sample size ≥ 25 . For laying hens only data from sampling during the production period were included. A mandatory *Salmonella* control programme for flocks of laying hens has been implemented since 2008. The discontinued trend line for *S. Enteritidis* in laying hens indicates that monitoring data from 2007 were from sampling schemes that were not harmonised.

Concluding remarks

- In Europe and in most other continents, *S. Enteritidis* is the serovar that is most commonly associated with egg contamination in most of the egg and farm surveys carried out, whereas *S. Typhimurium* is more commonly associated with laying hens and eggs in a small number of other countries worldwide.
- *Salmonella* serovars differ in their ability to cause contamination on the eggshell or the egg contents, with *S. Enteritidis* causing a higher rate of contamination of egg contents compared with other serovars.
- There is a non-linear (quadratic) relationship between infection prevalence in laying hens and the rate of eggshell contamination but a linear relationship between the rate of contamination of egg contents and the prevalence of infected laying hens in a flock, with eggshell contamination occurring at a much higher rate than that of egg contents. The relative contribution of egg shell contamination to the public health risk associated with eggs is unclear, but considered to be lower than that of internal contamination.
- The application of *Salmonella* control programmes at EU level has led to a clear decrease in the incidence of *S. Enteritidis* infection in laying hen flocks, and of human infections due to this serovar.

6. Epidemiological aspects of *Salmonella* in laying hens

6.1. Patho-biology of different *Salmonella* serovars in laying hens

In chickens, *Salmonella* spp. can cause three types of disease: fowl typhoid (*S. Gallinarum* biovar *Gallinarum*), pullorum disease (*S. Gallinarum* biovar *Pullorum*) and paratyphoid (caused by several serovars and subspecies of *Salmonella*, such as *S. Enteritidis*, *S. Typhimurium* and *S. Heidelberg*) (Shivaprasad et al., 2013). *Salmonella* serovars responsible for paratyphoid disease in chicken can cause foodborne infections in people (Rabsch et al., 2001; Foley et al., 2011).

6.1.1. Factors influencing *Salmonella* pathogenesis in chickens

There are numerous factors that influence the pathogenesis of *Salmonella* in chickens, such as serovar, strain, and route of infection (Shivaprasad et al., 2013).

S. Gallinarum is a non-flagellated (and therefore non-motile) serotype of *S. enterica* and is able to systemically spread in chicken. Both biovars *Gallinarum* and *Pullorum* are able to colonise the ovary and cause vertical transmission to the progeny (Uzzau et al., 2000). In the paratyphoid diseases, infection is largely confined to the gastrointestinal tract and is accompanied by faecal excretion (Shivaprasad et al., 2013). Some *Salmonella* serovars responsible for paratyphoid infection (such as *S. Infantis* and *S. Montevideo*) are less invasive but able to achieve a more extensive gastrointestinal colonization, therefore shedding larger numbers of bacteria over longer periods (Barrow et al., 1988). Other serovars (such as *S. Enteritidis* and *S. Typhimurium*) are more effective at spreading from the gastrointestinal tract to other organs, causing a systemic infection (Humphrey et al., 1989a; Keller et al., 1995; Okamura et al., 2001a; Okamura et al., 2010; Shivaprasad et al., 2013). In recent studies performing comparative genome analysis, it was concluded that *S. Gallinarum* and *S. Enteritidis* are highly related and that *S. Gallinarum* may be a direct descendant of *S. Enteritidis*, which has lost part of its genome and became host adapted. It was suggested that gene loss may be a mechanism of targeting the invading pathogen preferentially to particular tissues or host cells, and can also influence the ability of the pathogen to survive in the external environment or even in stressful situations within the host (Thomson et al., 2008; Feng et al., 2013). The enhanced invasion of the reproductive tract and survival in the forming egg of *S. Enteritidis* and *S. Gallinarum* has been linked to the presence of SEF-14 fimbriae ((Peralta et al., 1994; Thiagarajan et al., 1996; Rajashekara et al., 2000; Rank et al., 2009). *S. Enteritidis* can colonize the intestinal epithelium of chickens and is then able to reach a variety of internal organ sites, notably the ovaries (Gast and Beard, 1990; Gast, 1994; Peralta et al., 1994; Thiagarajan et al., 1996; Rajashekara et al., 2000; Rank et al., 2009; Berghaus et al., 2011). Studies have shown that some *S. Enteritidis* phage types (PTs) are more invasive than others (Hinton et al., 1990; Roy et al., 2001), but other similarly designed studies could not identify any difference in the invasiveness or colonisation ability between different phage types (Barrow, 1991). Despite its peculiar affinity for the reproductive tract of hens, *S. Enteritidis* is not the only serotype able to colonise ovaries and oviducts and contaminate forming eggs (Keller et al., 1997; Okamura et al., 2001a; Gast et al., 2004; Gantois et al., 2008).

S. Typhimurium strains can be further subdivided into definitive phage types (DTs), according to their susceptibility to a series of bacteriophages (Anderson et al., 1977; Rabsch et al., 2002). Some *S. Typhimurium* DTs (such as DT104 and DT49) are able to infect a broad range of animal species, while others are host adapted (such as DT2, DT40 and DT99 in wild birds and DT8 in ducks) (Rabsch et al., 2002). When *S. Typhimurium* types that are host adapted to other avian species infect chickens, they normally cause a short-lived infection (Martelli and Davies, 2012). Other *S. Typhimurium* DTs, such as DT104 and DT49, can infect chickens and cause egg contamination (Threlfall et al., 1990; Williams et al., 1998; Okamura et al., 2010). The virulence and invasiveness of *S. Typhimurium* are also determined by the bacterial strain (Barrow et al., 1987; Keller et al., 1997; Okamura et al., 2010; Wales and Davies, 2011). For example, a study investigating the invasiveness and egg contamination potential of 10 *S. Typhimurium* DT104 strains has shown that they differed in their ability to cause ovarian infection and egg contamination (Okamura et al., 2010).

Chicks are more susceptible to *Salmonella* infection by inhalation and parental routes than via the oral route (Barrow et al., 1987; Baskerville et al., 1992; Cooper et al., 1994; Shivaprasad et al., 2013). *In ovo* infection occurs more frequently with serovars such as *S. Gallinarum* and *S. Enteritidis*, due to their particular affinity for the avian reproductive tract (EFSA BIOHAZ Panel, 2010b). The ability of *S. Enteritidis* to cause vertical transmission and infect chicken embryos is considered one of the reasons for the worldwide spread of this serovar in the chicken population during the 1980s (Thorns, 2000).

6.1.2. Resistance and immune response of poultry to *Salmonella* with special regard to laying hens

The immune response against *Salmonella* infection is both innate and acquired. When *Salmonella* first reaches the intestine, it invades the intestinal epithelium, rapidly attracting immune cells (such as polymorphonuclear leucocytes and macrophages) to the site (Van Immerseel et al., 2005). More than 90 % of the *Salmonella* cells that invade beyond the intestinal mucosa are destroyed by these phagocytic cells. *Salmonella* has adapted to grow inside host macrophages, which are therefore also sites of bacterial multiplication and a vehicle for systemic distribution of *Salmonella* via the lymphatic and blood circulatory systems to other organs (Uzzau et al., 2000). Paratyphoidal infections in chickens are largely restricted to the intestinal lumen and evoke an acquired immune response, which mainly involves the production of immunoglobulin (Ig) A (as it can be secreted across intestinal epithelia and into the lumen). Clearance of primary *S. Enteritidis* and *S. Typhimurium* infection is dependent on age of the chicken and host genetics (Beal and Smith, 2007). Cell-mediated immunity plays a more important role than the humoral response in protection and against *Salmonella* infection (Van Immerseel et al., 2005).

Wigley et al. (2005) have demonstrated that at the onset of laying both the T-cell response to *Salmonella* and non-specific responses to mitogenic stimulation fall sharply in both infected and non-infected birds. The fall in T-cell responsiveness coincides with the increase in numbers of *S. Gallinarum* biovar Pullorum and its spread to the reproductive tract. Three weeks after the onset of egg laying, T-cell responsiveness began to increase again and bacterial numbers declined. Specific antibody levels changed little at the onset of lay but increased following the rise in bacterial numbers in a manner reminiscent of a secondary antibody response to re-challenge. These findings indicate that a non-specific suppression of cellular responses occurs at the onset of lay and plays a major role in the ability of *Salmonella* to infect the reproductive tract, leading to transmission to eggs (Wigley et al., 2005).

6.1.3. *Salmonella* vaccination in chickens

The first experiences with successful vaccines against *Salmonella* in poultry were gained in immunization against fowl typhoid. Such vaccines are not required or recommended in industrialized countries where the infection has been eradicated or occurs only infrequently, but they are recommended in countries where the disease is still prevalent. During the last three decades, several experimental and commercial (live oral or injectable killed) vaccines have been described as successfully reducing the spread of infection and clinical disease ((Barrow et al., 1990; Griffin and Barrow, 1993). Among them the most established vaccine is the 9R strain of *S. Gallinarum*, which is also commercially available (Gupta and Mallick, 1977).

Vaccination of laying hens against *S. Enteritidis* and *S. Typhimurium* has been shown to confer protection against *Salmonella* infection and to decrease the level of on-farm contamination (Van Immerseel et al., 2005). Vaccination of flocks of laying chickens started in some countries during the 1990s in response to the *S. Enteritidis* pandemic (Thorns, 2000). As *S. Enteritidis* and *S. Typhimurium* are considered to be most the important serovars for public health in Europe, existing commercially available live and inactivated *Salmonella* vaccines for poultry are intended for use against one or both of these serovars. In some European countries (Austria, Belgium, The Czech Republic, Germany and Hungary) vaccination of laying flocks is compulsory, in others vaccination is permitted and recommended (Bulgaria, Belgium, Cyprus, Estonia, France, Greece, Italy, Latvia, Lithuania, The

Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, and the United Kingdom) while in others it is banned (Denmark, Finland, Sweden and Ireland) (Galiş et al., 2013).

Vaccination is used to prevent systemic infection (and localization in the reproductive tract) and to reduce faecal shedding (and consequently carcass and/or egg contamination). Vaccination is regarded only as an additional measure to increase the resistance of chicks to *Salmonella*, especially if the flock prevalence is high. Although such vaccination is not fully protective, especially in the case of laying hens placed in a previously contaminated laying house, it is likely to reduce faecal shedding, ovarian transmission, and the within-flock prevalence, thereby reducing contamination of table eggs and the environment. Most importantly, the use of vaccination against *S. Enteritidis* and *S. Typhimurium* seems to lower internal-egg contamination levels, thereby most directly contributing to public health (Davies and Breslin, 2004; Gantois et al., 2006). In the EU-wide baseline study conducted in 2004-2005, vaccination of laying flocks was found to decrease the risk of *S. Enteritidis* compared with unvaccinated flocks. Vaccination was demonstrated to be particularly effective in MSs with high farm prevalence (more than 15 %) of *Salmonella* infection (EFSA, 2007a).

There is some indication in the literature that eggs laid by vaccinated chickens may be more resistant to *Salmonella* contamination and further multiplication, as maternal anti-*Salmonella* antibodies can be present in the egg (Hassan and Curtiss, 1996). In a recent study conducted in broilers, the samples collected from flocks that were progeny of vaccinated broiler breeders had a 62 % lower chance of being *Salmonella* positive than samples collected in equivalent flocks that were progeny of unvaccinated breeders (Berghaus et al., 2011).

6.2. Dynamics of *Salmonella* infection in the laying hen flock

Infection dynamics of *S. Enteritidis* may depend on a number of factors (Howard et al., 2012) e.g. housing system, flock management, breed of hen, *Salmonella* strain, time of exposure, presence of vectors, etc. Several epidemiological studies have been published, among them observational studies aiming to identify risk factors for introduction of *Salmonella* in laying hen flocks. Risk factors concerning flock characteristics were flock size (Mollenhorst et al., 2005; EFSA, 2007b; Namata et al., 2008; Huneau-Salaun et al., 2009), and flock age (Garber et al., 2003; EFSA, 2007b; Namata et al., 2008). The size of the farm is also linked with hygiene practices, as large farms are more likely to be dry cleaned only, rather than washed and disinfected, between flocks (Aimey et al., 2013). Also, farm management and type of housing were identified as risk factors. Both on-floor systems (Garber et al., 2003; Mollenhorst et al., 2005) and cage systems (EFSA, 2007b; Namata et al., 2008; Gast et al., 2013) were found to increase the risk of colonization of flocks by *Salmonella* or of egg contamination by these or other bacteria (De Reu et al., 2009; Jones et al., 2012), including *Campylobacter* (Messelhäuser et al., 2011), in different studies, or to have no influence in others. These contrasting findings are likely to relate to national variations in housing systems, management and sources of birds, but, in general, cage production has been found to be associated with an increased risk of flock infections by *Salmonella*, and non-cage systems result in dirtier eggs, which are more likely to be contaminated by pathogens if the flock is infected (Holt et al., 2010). The age of the poultry house is also a significant risk factor, as most *S. Enteritidis* infections have persisted in laying farms for decades after their original introduction with birds from infected breeding flocks in the 1970s and 1980s (Van Hoorebeke et al., 2010). Multi-stage management in on-floor flocks was also identified as a risk factor ((Mollenhorst et al., 2005; Huneau-Salaun et al., 2009) and the finding of generally lower risk probably relates to the greater use of all-in/all-out systems and smaller numbers of flocks and birds in non-cage systems.

Other factors were the occurrence of *Salmonella* in the previous flock in floor-housed flocks (Huneau-Salaun et al., 2009), the absence of cleaning and disinfection of the poultry house between subsequent production cycles (Garber et al., 2003) and entry of delivery trucks near the poultry house entrance (Huneau-Salaun et al., 2009). In addition, it appeared that seasonality was associated with serotypes other than *S. Enteritidis* and *S. Typhimurium* (EFSA, 2006). Vaccination of hens against *Salmonella* was a protective factor, except for *S. Typhimurium* (EFSA, 2007b). Continued production of heat-

treated eggs from infected flocks, rather than culling, is associated with greater long-term persistence of *S. Enteritidis* on laying farms and more limited progress in reducing the national flock prevalence (Dewaele et al., 2012). Leaving infected flocks in production also provides a localized reservoir of *S. Enteritidis* that can be disseminated more widely via manure, etc. (Wales and Davies, 2013).

Specific risk factors associated with *Salmonella* contamination of eggshells in one study were entry of delivery trucks near poultry house entrance, flock size, heavily contaminated environment, high egg laying rate and mixed farming (Chemaly et al., 2009).

New EU welfare legislation has led to a ban on the use of conventional 'battery' cages for laying hens since January 2012, resulting in cage houses having to be either decommissioned or refurbished to provide an alternative housing system. Such alternative systems that involve smaller flocks are less conducive to *Salmonella* infection. Some cage houses may be converted to barn production, typically as two-storey barns, but the most likely option is conversion to enriched colony cages, in which groups of 30–80 birds are housed in a larger cage that provides more space, perches and a 'nest-box' area. Conversion of houses required mass removal of old-style cages (Van Hoorebeke et al., 2011) and this offered an excellent opportunity to eliminate farm pests that can carry *Salmonella*, such as rodents, flies and litter beetles, as well as red mites, which can reduce the resistance of birds to *Salmonella* infection in the case of heavy infestations. During the extended down-time involved in refurbishment, houses can be deep cleaned and intensively disinfected to remove residual environmental contamination. This was a great opportunity to eliminate resident *Salmonella* from cage houses that should reduce the infection risk (van Hoorebeke et al., 2012). Colony cage nest and perch areas can be more difficult to clean than conventional cages but the belt-cleaning system means that there is less harbourage for rodents and flies than in deep pit houses that they replace. Despite the fact that numerous risk factors associated with colonization have been identified and quantified, and several control measures have been implemented (Galiş et al., 2013), introduction of *Salmonella* into flocks may still occur, albeit at a lower frequency than before (van de Giessen et al., 2006). Some of the cases of apparent new infections may also be examples of chance detection of infection that was previously below the limit of detection.

After introduction into a flock, typically via persistent contamination of the laying house or in replacement birds or wildlife vectors (Howard et al., 2012; Wales and Davies, 2013), transmission of *Salmonella* between hens occurs via contact with infected individuals and ingestion of faecally contaminated materials (Holt et al., 1998), feed and water (Nakamura et al., 1994; Holt, 1995; Nakamura et al., 1997), and aerosols/dust (Baskerville et al., 1992; Nakamura et al., 1997; Gast et al., 1998; Holt et al., 1998). Dust is also relevant to the contamination of eggs by *B. cereus* group (Koné et al., 2013). The potential for contact transmission of *Salmonella* may be greater when birds are subjected to stress, especially induced moulting (Holt and Porter, 1992; Holt, 1995; Holt et al., 1998). All studies mentioned above, however, were carried out with hens housed in wire-floored cages and used high experimental doses.

After colonisation, individual laying hens shed *Salmonella* in their faeces intermittently, as measured by routine culture methods. Most hens stop shedding the bacteria after approximately three weeks (Shivaprasad et al., 1990; Gast, 2005). However, under stress (water deprivation, viral or coccidial infection, stressful environments and moulting) the hens may resume shedding (Skov et al., 2002). This can be explained by reactivation of shedding in latent carriers (Barrow, 1992) or by a higher susceptibility to re-infection from the environment (Skov et al., 2002) as *S. Enteritidis*, in particular, has a tendency to show long-term persistence in laying houses, possibly related to rodent levels and housing system (Carrique-Mas et al., 2008). In most poultry houses with vaccinated flocks, *S. Enteritidis* and other serovars do not persist once rodents are eliminated (Davies and Carrique-Mas, 2010).

Important parameters used for quantification of transmission are the basic reproduction ratio R_0 , defined as the average number of secondary cases caused by one typical infectious case in a fully susceptible population, and the transmission rate parameter β , defined as the number of new infections

that occur due to one infectious animal per unit of time. In field studies, the level of infection is commonly higher at the end of the laying period, although there can also be an early peak soon after the introduction of birds into the laying house (Davies and Carrique-Mas, 2011; Bouzidi et al., 2012), but detailed longitudinal studies on within-flock prevalence, ideally with quantification of shedding, are required to fully elucidate flock infection dynamics in actual farm conditions.

Concluding remarks

- The ability of *S. Enteritidis* to persist in the ovaries and oviduct of chickens and cause vertical (ovarian) transmission is considered one of the main reasons for the worldwide spread of this serovar in chicken populations during the 1980s.
- Vertical transmission leads to the internal contamination of forming eggs, as well as shell contamination. This, together with the wide distribution and persistence of *S. Enteritidis* in European laying flocks before harmonised control programmes were introduced, has led to this serovar becoming predominant among food-borne *Salmonella* infections.
- Certain strains of other serovars, such as *S. Heidelberg*, *S. Typhimurium* and *S. Infantis* can also infect laying hens and contaminate eggs, but internal contamination is not as great as for *S. Enteritidis*.
- Vaccination of laying hens against *S. Enteritidis* is considered to be the most effective measure for reduction of egg-borne infection in humans, although it does not result in full protection of flocks.
- The replacement of deep pit cage houses with colony cages or alternative housing systems is thought to have had a beneficial effect in terms of reducing the persistence of *Salmonella* on laying farms.
- A control measure involving heat treating the eggs from infected flocks rather than culling birds is associated with greater long-term persistence problems of *S. Enteritidis* on laying farms and more limited progress in reducing the prevalence in the national flock.

Recommendations

- Vaccination of laying hens against *S. Enteritidis* should be maintained in those MSs that previously had a flock prevalence of more than 10 % in the EU baseline survey, unless it can be shown by sensitive monitoring that current infection levels are extremely low.
- There should be continuous scrutiny of surveillance data from breeding flocks and commercial laying flocks, as well as from human egg-borne *Salmonella* outbreaks, so that new and emerging strains of *Salmonella* with the potential for significant vertical transmission can be detected and controlled at an early stage.

7. Detection of *Salmonella* in laying hen flocks and eggs

7.1. Farm level

7.1.1. Sampling methods

The efficiency of sampling programmes has a large impact on the detection of *Salmonella* and therefore estimation of prevalence (Fletcher, 2006). It has been recognised for some time that thorough environmental sampling is the most effective way to detect zoonotic serovars of *Salmonella* in a poultry flock (Aho, 1992; Johansson et al., 1996; Musgrove and Jones, 2005).

Boot or sock swabs are the easiest method for obtaining floor faecal samples from non-cage units, but pooled faecal droppings samples are used for caged flocks. Large hand-held gauze (or ‘chiffonette’) swabs can also be effective for sampling (Davies and Wray, 1996; Carrique-Mas and Davies, 2008; Zewde et al., 2009) but more effort and dedication are required to achieve a representative sample.

Dust is a useful sample for identifying previous excretion of *Salmonella* by a poultry flock, especially in cage houses ((Riemann et al., 1998; Arnold et al., 2014b). It is normally best to take both fresh faecal and dust samples (Davies and Wray, 1996) to help compensate for variable detection in either sample. In the EU Baseline survey of laying hens for *Salmonella* in 2004/2005, only one country, Ireland, had better detection of infected flocks via national monitoring than in the survey, and this was because routine sampling in that country was based on dust.

7.1.2. Detection methods

A standard culture method, ISO 6579, Annex D (Amendment 1 of EN/ISO 6579-2002/Amd1:2007. 'Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Salmonella* spp. — Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage) is used in the EU for samples from primary production. Rapid detection methods such as PCR, gene probes and enzyme-linked immunosorbent assay (ELISA)-based tests have been described for use with poultry samples, but can be more subject to interfering substances in the sample (Jensen et al., 2013) and inter-laboratory variation. So far no alternative methods have been approved for statutory use for monitoring food-producing animal populations in the EU, but several have been authorised in the USA (Adams et al., 2013).

Immunological detection by serology can also be used to identify indirect evidence of likely exposure to *Salmonella* by detecting antibodies in serum or egg yolk (Davies et al., 1997; Feld et al., 2000). This increases the sensitivity of detection of those serotypes whose surface antigens are included in the ELISA-based test, normally *S. Enteritidis* and *S. Typhimurium*, compared with bacteriology alone, and a combination testing programme has been successfully used in Denmark for many years (Wegener et al., 2003). Such testing cannot readily be used in vaccinated flocks but is a useful additional voluntary measure in non-vaccinated flocks. Serological testing frequently detects false-positive reactions (Klinkenberg et al., 2011) caused by exposure of birds to organisms with antigens that are shared with the target organisms so they can only be used as an adjunct to bacteriological monitoring.

7.2. Eggs

7.2.1. Sampling of eggs

The main difficulty with sampling of eggs to detect *Salmonella* is the very low expected prevalence, especially in relation to egg contents. It is necessary to sample 3 000 eggs in order to reliably detect the expected 0.1 % prevalence of positive egg contents from an infected flock, and 4 000 whole eggs (including shells), pooled in lots of 40, has been designated as an additional voluntary confirmatory sample for laying flocks for which a false-positive flock faecal sample is suspected (Regulation (EC) No 1237/2007²⁴). Samples taken from egg conveyor, candling and grading systems and the floor beneath such handling equipment provide a more sensitive indication of the prior passage of contaminated eggs. Gentle homogenisation of egg contents prior to culture in the laboratory increases the chance of detection, as it makes the yolk contents available to the bacteria.

7.2.2. Detection methods in eggs

7.2.2.1. Culture methods

Because of the typically low prevalence of contaminated eggs, the low numbers of organisms in such eggs and the bacteriostatic effect of the albumen, multiplication of these relatively few organisms is necessary to reach detectable levels in culture. This can be achieved using traditional three-step *Salmonella* culture methods. Longer periods of pre-enrichment (48 hours rather than 24 hours) have been shown to further increase the sensitivity of detection in individual eggs or in pooled egg contents, as long as only a few competing organisms are present. In practice, in the EU, eggs are most likely to

²⁴ Commission Regulation (EC) No 1237/2007 of 23 October 2007 amending Regulation (EC) No 2160/2003 of the European Parliament and of the Council and Decision 2006/696/EC as regards the placing on the market of eggs from *Salmonella* infected flocks of laying hens. OJ L 280, 24.10.2007, p. 5-9.

be tested as a foodstuff, therefore the full ISO 6579 method is used. Pre-enrichment at 41.5 °C may also be beneficial in some cases (Park et al., 2012).

In a recent study, (Pasquali et al., 2013) tested the effect of pooling and of the initial contamination level on the detection of *Salmonella* on table eggs by the reference method ISO 6579. The authors found that the testing sensitivity on pooled samples was affected by the initial level of *Salmonella* contamination in the single positive egg, but not by the dilution of the positive egg with increasing number of negative ones. It is concluded that at least 16 pooled samples of 10 eggs each, characterised by a low prevalence and low contamination level, need to be tested in order to detect *Salmonella* with 95 % certainty following the ISO 6579 method.

The albumen of the egg has a strong bacteriostatic effect due to the presence of ovotransferrin, which limits the amount of iron available to the bacteria. Iron supplementation can therefore help overcome this effect, enhancing the growth of *Salmonella* and therefore increasing the sensitivity of detection. This is especially necessary in situations in which abbreviated methods (i.e. incubation followed by direct plating) are used, rather than the three-step culture method using pre-enrichment. Iron salts such as ferrous sulphate have also been shown to promote the isolation of *S. Enteritidis* from egg contents and may replace the pre-enrichment step of the culture when added to the egg pool prior to incubation (Chen et al., 2001). However, iron salts may also stimulate the growth of competing bacteria. Ferrioxamines, on the other hand, act by supplying *Salmonella* with useable iron, rather than saturating ovotransferrin, and therefore do not promote the growth of *E. coli* and the *Proteus*–*Providencia*–*Morganella* group, provided that they are not supplied at high concentration.

7.2.3. Factors influencing detection of *Salmonella* infected flocks

The reports of the baseline survey, and other related papers (Methner et al., 2006; Much et al., 2007; Snow et al., 2007), have shown an increased risk of detection of *Salmonella*, mainly *S. Enteritidis*, in caged flocks. This may result from a less diverse intestinal microbiota and more persistent colonisation by *Salmonella* in confined small groups of birds (Nordentoft et al., 2011; Gast et al., 2013). Eggs are more likely to be contaminated when infected birds are placed in floor-based housing (De Vylder et al., 2011). Collect representative faecal samples in colony cage houses is much more difficult as the manure belts are often inaccessible and lack scrapers that accumulate faeces and stacks of cages can be very high, further limiting access for sampling. This means that it is now more likely that infection will be effectively detected in non-cage houses using two pairs of boot swabs than in colony cage houses using two composite faecal samples (Arnold et al., 2014b). The addition of dust to sampling programmes (Iwabuchi et al., 2010) is particularly valuable in colony cage houses, but is now rarely done by producers or official samplers in most MSs.

Several studies have shown an increased tendency for flocks to be identified as *Salmonella* positive as the birds become older (Garber et al., 2003; van de Giessen et al., 2006; Wales et al., 2007; Bouzidi et al., 2012; Roberts et al., 2013) especially if birds have been moulted (Golden et al., 2008). In most cases, the initial infection resulted from residual contamination of laying houses that spread to pullets that are suffering from transport, handling and relocation/remixing stress at a time when hormonal changes associated with the onset of lay are also increasing susceptibility to infection (Line et al., 1997). This leads to a typical early peak of infection within three weeks of housing (Humbert et al., 1995; Gradel et al., 2002) but laying flocks are rarely sampled at this time (16–19 weeks of age). There may also be an increase in excretion towards the end of lay, but in the absence of rodents this is less likely to occur and infection may spontaneously resolve (Carrique-Mas et al., 2009).

A range of potential food poisoning bacteria such as *E. coli*, *Klebsiella* spp., the *B. cereus* group, *S. aureus*, *C. perfringens* and *Yersinia enterocolitica* may be found in the faeces of laying hens and on eggshells. There are not likely to present a significant hazard in relation to use of fresh eggs, but shell contamination may result in the introduction of contamination into egg-processing facilities. An Australian study found that there was no significant difference in total Enterobacteriaceae count on the eggs of the flock from early-, mid- or late-lay flocks. Enterobacteriaceae isolates were of 11 different

genera: *Cedecea*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Pantoea*, *Salmonella*, *Serratia* and *Yersinia*. Out of all 153 identified Enterobacteriaceae isolates, the *Escherichia* genus was reported most frequently (60.8 %) (Gole et al., 2013).

Both live and inactivated vaccines are available for the control of *S. Enteritidis* and/or *S. Typhimurium*. The protection offered by vaccination is often not complete or sustained, although the likelihood of infection of eggs is reduced (Davies and Breslin, 2003). Detection of infected flocks may also be reduced as a result of reduction of the within-flock prevalence (Berghaus et al., 2011) and number of organisms shed in faeces (Van Immerseel et al., 2004; Gantois et al., 2006; Inoue et al., 2008). Waning of vaccinal protection may be involved in the rise in excretion towards the end of lay.

Overall, it is clear that detection of *Salmonella* in laying flocks is far from straightforward, and some positive ones will not be detected by any method, including the baseline survey and control programme methods that are used for confirmatory testing (Zenner et al., 2013; Arnold et al., 2014b). Since none of the sampling methods has a high level of sensitivity, any confirmatory sampling may possibly negate a previous positive result, even if there is no interference with the process by actions taken by the operator. A comparison of operator and official sample results provides an indication of some of the likely testing deficiencies in monitoring programmes (Arnold et al., 2010; Arnold et al., 2014b) that might lead to under-detection of infected flocks. The significance of under-detection in terms of public health is unclear, as it is the most highly infected flocks that are likely to be detected, and eggs from flocks with low levels of infection are less likely to be contaminated (Van Hoorebeke et al., 2009), but there is still some way to go before *S. Enteritidis* infections in the European population reach the low levels of the pre-epidemic period in the 1970s.

7.2.4. Factors influencing detection of *Salmonella* in eggs

The main challenge concerning the detection of *S. Enteritidis* in eggs is the typically very low rate of egg contamination, even among eggs originating from *S. Enteritidis*-infected flocks. In addition, only very few *S. Enteritidis* organisms are deposited within contaminated eggs. There is, however, a linear relationship between within-flock prevalence and the rate of contamination of egg contents as well as a more substantial contribution of prevalence to shell contamination (Arnold et al., 2014a). In many countries, flocks of commercial laying hens are vaccinated against *S. Enteritidis*, which may further reduce the rate of contamination in eggs. These factors make the detection of *S. Enteritidis* in raw eggs a challenging task. In most situations, in order to avoid overwhelming laboratory resources, the contents of between 6 and 40 eggs are pooled and cultured together. There might be some reduction in the test sensitivity due to a dilution effect derived from pooling eggs (Humphrey and Whitehead, 1992), although the precise magnitude of this effect is unknown. Because direct plating would not be able to detect fewer than 10^4 CFU/mL of *Salmonella*, additional steps or enhancements to the culture procedures are necessary. Pools of egg contents are either incubated or pre-enriched (or both) with or without supplementation of additives that promote the growth of *Salmonella* under the iron-limitation conditions that apply in egg albumen.

Concluding remarks

- Detection of *Salmonella* in laying flocks is far from straightforward and some infected flocks will not be detected by any routine sampling method.
- The addition of dust samples to testing programmes increases the sensitivity of detection of infected flocks.
- The sensitivity of the sampling programme carried out using current harmonised monitoring programme for *Salmonella* in laying hens is limited. This may involve difficulties in representative sampling, particularly in large colony cage houses, poor sample handling and deficiencies in laboratory testing standards or deliberate interference.
- The application of confirmatory sampling in many MSs may allow some infected flocks to remain in production.

- Detection of *Salmonella* in eggs is difficult because of the low prevalence of positive eggs, even in ones from most infected flocks, and the inhibitory effect of egg albumen.
- Samples taken from egg conveyors and grading equipment provide more sensitive detection of contamination of batches of eggs that have been handled.
- A range of potential food poisoning bacteria, such as *E. coli*, *Klebsiella* spp., the *B. cereus* group, *S. aureus*, *C. perfringens* and *Y. enterocolitica*, may be found in the faeces of laying hens and/or on egg shells. These are not likely to present a significant hazard in relation to use of table eggs but shell contamination may result in the introduction of contamination into egg-processing facilities.

Recommendations

- The difference between the results of official testing of laying flocks and operator testing in flocks of laying hens across Europe could be analysed in detail to investigate the limitations of test sensitivity.

8. Mechanisms of primary and secondary contamination of eggs by *Salmonella* spp. and possible cross-contamination along the food chain

Salmonella spp. can contaminate egg contents and membranes, or the outer surface of the eggshell. If *Salmonella* contamination occurs before shell formation, it is defined as ‘primary’ contamination (also called vertical or ovarian transmission). If it occurs after the egg has been laid through eggshell penetration, it is defined as ‘secondary’ contamination (Methner et al., 1995). For the purpose of this opinion, cross-contamination is considered to have occurred when the eggshell becomes contaminated through contact with contaminated surfaces such as egg-handling equipment. The contamination that may occur during food preparation or the production of egg products is also referred to as cross-contamination.

8.1. Primary contamination

Primary contamination occurs when the egg is contaminated by *Salmonella* during its formation. Several studies have demonstrated that *Salmonella* can colonize the reproductive tract and colonize eggs (Gast, 1994; Keller et al., 1997; Okamura et al., 2001a; Okamura et al., 2001b).

S. Enteritidis is the serovar most frequently associated with egg infection (EFSA BIOHAZ Panel, 2010a)). This is due to two main factors: its unique ability to colonize the ovary and oviduct of laying hens long-term; and its spread and persistence in the parental breeder flock population in most of the world (Thorns, 2000).

S. Enteritidis is particularly successful in invading the hen reproductive tract and contaminating eggs (Humphrey, 1994; Okamura et al., 2001a; Okamura et al., 2001b; Gast et al., 2004; Gantois et al., 2008). The success of *S. Enteritidis* in the transovarian transmission route can be associated with the presence in this serovar of the SEF14 fimbriae (which might be involved in the colonization of the reproductive organs), the presence of the *yafD* gene (which is essential for resistance in the albumen), and with the lipopolysaccharide structure, a key factor in oviduct persistence and survival in egg albumen (Lu et al., 2003; Coward et al., 2013), particularly at a hen body temperature of 41.5 °C (Coward et al., 2013). *S. Typhimurium* is able to colonize ovaries and internally contaminate eggs (Okamura et al., 2001a; Wales and Davies, 2011; Martelli and Davies, 2012). Other *Salmonella* serovars are rarely isolated from egg contents, but can infect the hen reproductive organs (Okamura et al., 2001a; Gast et al., 2004; Martelli and Davies, 2012). In an intravenous infection study, *S. Heidelberg* was demonstrated to be able to survive in the albumen during egg formation, while *S. Virchow* and *S. Hadar* were eliminated more rapidly, suggesting that, in natural infections, they might be killed in the albumen before ovodeposition (Gantois et al., 2008).

Salmonella egg content contamination has been observed most commonly on the yolk membrane (Humphrey et al., 1989a; Timoney et al., 1989; Shivaprasad et al., 1990; Gast and Holt, 2000, 2001; Gast et al., 2002; Gast et al., 2013) or in the albumen (Humphrey et al., 1989a; Gast and Beard, 1990; Shivaprasad et al., 1990; Gast and Beard, 1992; Thorns, 2000). Direct contamination inside the yolk is also possible but it is a rare event (Gast and Holt, 2001; Gast et al., 2003).

Salmonella can colonize different sections of the reproductive tract, and the deposition site in the egg depends on the site of colonization (Gantois et al., 2009). Yolk contamination points to the ovary as the site of origin of the egg contamination (De Buck et al., 2004). A systemic spread of *Salmonella* infection in the hen can lead to the colonization of the ovary (Keller et al., 1995) and *Salmonella* (in particular *S. Enteritidis*) is strongly believed to interact with the cellular components of the preovulatory follicles, which lead to the production of the egg yolk. Ovary contamination with *Salmonella* can result in colonization of the preovulatory follicles, but not necessarily in the production of yolk-contaminated eggs. As the follicles are particularly rich in nutrients, *Salmonella* grows extensively in them at a chicken body temperature of 42 °C, and this can lead to degeneration of the contaminated follicle before the production of the egg (Gantois et al., 2009). In studies involving experimental infections of laying hens, the presence of *Salmonella* in the ovaries did not necessarily correspond to the production of contaminated egg contents (Barrow and Lovell, 1991; Keller et al., 1997).

Salmonella infection of the oviduct can occur and result in contamination of the albumen, eggshell membranes and eggshell itself, depending on the site of colonization (magnum, isthmus and uterus, respectively) (Gantois et al., 2009). In the majority of the eggs infected via the oviduct tissue, *Salmonella* is deposited in the albumen (Gast, 1994; Gast and Holt, 2000). For example, Shivaprasad and colleagues (1990) observed that challenge with *S. Enteritidis* PT8 resulted in egg content contamination and that albumen contamination occurred (at frequencies as high as 2.9 %) more often than that of the yolk (≤ 0.7 %). Forming eggs have been shown to have a higher contamination rate than laid ones, as the egg albumen contains antibacterial compounds that reduce the bacterial load over time (Keller et al., 1995). *Salmonella* cannot replicate effectively in the albumen of fresh eggs, and therefore must gain access to the yolk contents in order to multiply (Gantois et al., 2008). The vitelline membrane in fresh eggs inhibits yolk invasion by *Salmonella*, but gradually this deteriorates during storage, leading to penetration in the egg yolk (Humphrey and Whitehead, 1993). Migration of *Salmonella* from the albumen to the yolk during storage positively depends on the contamination dose, the temperature, the age of the eggs and the frequency of migration (Braun and Fehlhaber, 1995).

The hen lower reproductive tract (isthmus, shell gland and vagina) produces the egg membranes and eggshell. It can become contaminated with *Salmonella* either via descending infection from the bloodstream and the ovaries or via ascending infection from the cloaca. Therefore eggshell and membranes can become contaminated during egg development (De Buck et al., 2004). Several studies have reported positive eggshells and membranes in eggs produced by infected hens (Bichler et al., 1996; Miyamoto et al., 1997; Okamura et al., 2001b). However, as *Salmonella* can penetrate the eggshell (see Section 8.2) it is difficult to distinguish between contamination during egg formation and that occurring after ovodeposition (De Buck et al., 2004).

Little is known about the number of cells in an internally contaminated egg at time of lay, but it generally ranges from 1 to 400 *Salmonella* bacteria, with most eggs containing fewer than 40 *Salmonella* cells (Humphrey et al., 1989a; Gast and Beard, 1990; Humphrey et al., 1991; Gast and Holt, 2000). In a study of eggs produced by hens naturally infected with *S. Enteritidis*, the contents of all positive eggs were found to contain fewer than 10 *Salmonella* cells per egg (Humphrey et al., 1989a). In another study conducted on eggs from flocks naturally infected with *S. Enteritidis* PT 4, 72 % of the eggs contents found to be *Salmonella* positive (23/32) contained fewer than 20 cells of *S. Enteritidis* (Humphrey et al., 1991).

8.2. Secondary contamination

Salmonella can gain access to the contents of the egg by penetrating the eggshell (trans-shell contamination). A comprehensive review on the structure of the eggshell can be found in a previous EFSA opinion (EFSA, 2009b). Briefly, the egg has three barriers to bacterial penetration: the cuticle, crystalline eggshell and shell membranes (Gantois et al., 2009). Physical disruptions of these barriers facilitate bacterial penetration inside the egg. Cracks can be caused by, for example, poor egg collection management systems (De Reu et al., 2009) or rapid cooling of eggs (microcracks) (EFSA, 2009b). The age of the eggs and poor eggshell quality (for example, in eggs laid by older hens) are intrinsic factors that facilitate the penetration of *Salmonella* through the eggshell. Extrinsic factors, such as temperature, moisture and relative humidity can influence the penetration of bacteria (Messens et al., 2005; De Reu et al., 2006a). In particular, when a positive temperature differential is created between the egg (warmer) and the environment (cooler), bacteria migrate more easily through the eggshell and membranes. This can occur when the egg is just laid and the cuticle is immature (Bruce and Drysdale, 1994). Trans-shell contamination is not a unique property of *S. Enteritidis*, but other *Salmonella* serotypes and completely unrelated bacteria can penetrate the egg through this route (De Buck et al., 2004; De Reu et al., 2006c; Gantois et al., 2008). There is a positive correlation between the level of bacterial contamination on the eggshell and shell penetration and whole egg contamination by *Salmonella* (De Reu et al., 2006c).

8.3. Cross-contamination

Salmonella spp. are also found on the surface of the eggshell, and this can also be associated with internal egg contamination (De Buck et al., 2004; Gantois et al., 2009; Martelli and Davies, 2012). Faecal contamination of the eggshell is unlikely to occur during oviposition in a healthy laying hen, as the laying of the egg everts the vagina beyond the alimentary tract, protecting the egg from faecal contamination (De Buck et al., 2004). After deposition, the egg can come into contact with contaminated surfaces, such as nest boxes or collection belts, or contaminated feed or water (EFSA, 2009b). If the eggshell comes into contact with contaminated moist organic material, this facilitates the growth and survival of *Salmonella* by providing nutrients and a certain degree of physical protection (Gantois et al., 2009). *Salmonella* can survive on the eggshell in the absence of faecal material, especially at lower temperatures and low relative humidity (Messens et al., 2006). This seems to be related to the slower metabolism induced by the disadvantageous conditions on the dry eggshell surface (Gantois et al., 2009).

Cross-contamination along the food chain between surfaces and eggs can also lead to *Salmonella* contamination of the eggshell. Contamination in egg-packing plants may be a significant contributory factor to external contamination of eggshells. This has been shown with sterilized eggs being processed in packing plants and the critical points are the candling, grading and packing area (Davies and Breslin, 2003). There is some evidence of cross-contamination as the cause of *Salmonella* egg-borne outbreaks (Thomas et al., 2006; Roberts-Witteveen et al., 2009). Cross-contamination that involves other (raw or ready-to-eat) foods can occur also at home or in restaurants and the catering sector (Holtby et al., 1997). The levels of *Salmonella* egg surface contamination can be higher than those inside the freshly laid egg and this represents a cross-contamination risk for contamination of kitchen surfaces and in places where eggs are broken up (Luber, 2009).

The survival and growth of *Salmonella* on the eggshell and egg contents is dependent on deposition site, time and temperature, and is described in Section 11.2.

Concluding remarks

- *Salmonella*-infected hens can produce *Salmonella*-contaminated eggs. The number of contaminated eggs (particularly internally contaminated eggs) produced by a *Salmonella* infected flock is, however, very limited.
- The site of deposition of *Salmonella* within the egg depends on the stage of the egg formation process. If *Salmonella* is deposited before the shell is formed, the egg contents are contaminated. Contamination of the egg albumen is much more frequent than the contamination of the egg yolk.
- The number of *Salmonella* organisms in contaminated contents of fresh eggs is normally low (fewer than 20 cells), and increases significantly only if *Salmonella* reaches the nutrient-rich vitelline membrane and the yolk or when the yolk membrane degrades and releases nutrients into the albumen.
- Secondary and cross-contamination can lead to the contamination of the egg contents. The likelihood of contamination depends on the level of hygiene of the eggshell and on handling of eggs in processing plants and in the kitchen.

9. Structure and defence mechanisms of the egg against microbial contamination

The egg has several physical and chemical defence mechanisms that protect its contents from microbial invasion and multiplication. While the eggshell and shell membranes physically hinder microbial penetration into the egg albumen, the vitelline membrane reduces penetration into the most nutritious compartment of the egg, the egg yolk. The chalazae also act as physical barriers against bacterial penetration into the egg yolk by maintaining the yolk in a central position. Finally, the various antimicrobial properties of egg albumen, its viscosity, heterogeneity and alkaline pH inhibit bacterial proliferation and contribute to hindering egg yolk invasion.

9.1. The eggshell and cuticle

The eggshell is a highly ordered and mineralised structure mostly composed of calcium carbonate (CaCO_3) in the form of calcite. The eggshell is made up of five layers, i.e. a mammillary layer, cone layer, palisade layer, vertical crystal layer and cuticle (inner–outer). The purpose is to allow the exchange of water and gases between the exterior and the developing embryo while forming a physical barrier against external microbial contamination, as well as to provide calcium for embryo development when the yolk is depleted (Nys et al., 2004). Eggshell damage is directly related to shell strength, itself influenced by shell thickness and shell matrix organization. It decreases with the age of the hens in commercial laying flocks.

Thousands of pores are present in the shell; they are 12–20 μm in diameter and their number varies according to egg size and location (Bruce and Drysdale, 1994). The influence of eggshell porosity on microbial penetration is rather controversial in the literature; while some papers report a significant effect (Tyler, 1956; Fromm and Monroe, 1960; Board and Halls, 1973), others refute this view (Nascimento et al., 1992; De Reu et al., 2006c). The cuticle is an uneven layer whose inner part is made up of a protein–carbohydrate complex containing a small amount of the crystal complex hydroxyapatite deposited during the final phase of eggshell calcification; the outermost layer remains non-mineralized (Rose-Martel et al., 2012). Its function is either to bridge the outer pore openings or to extend down into the pore canals, plugging them (Board, 1982). The cuticle also links the lumina of pore canals to the egg's exterior and, thereby, serves as a pathway for gas diffusion (Board and Scott, 1980). Numerous factors such as the age of the animal, feeding regime, number of eggs laid, etc., influence the extension and composition of the cuticle (Nascimento et al., 1992; Messens et al., 2007).

9.2. The shell membranes

The shell membranes contribute to the shell strength by serving as a reinforcement of the inner parts of the eggshell. They are very effective barriers to bacterial penetration and at the same time have

antibacterial properties (Gautron et al., 2001a; Gautron et al., 2001b; Gautron et al., 2011). The shell membranes are built up of three distinct layers: (i) the inner shell membrane (ISM; lies immediately over the albumen); (ii) the outer shell membrane (OSM; attached to the eggshell), which consists of a network of randomly oriented fibres; and (iii) a homogeneous third layer of electron-dense material called the limiting membrane (Bruce and Drysdale, 1994). This limiting membrane intermeshes with the innermost region of the inner membrane fibres rather than forming a separate and distinct layer (Wong Liong et al., 1997). Shell membranes are made up of fibrous material or membrane fibres whose diameter ranges from 0.4 to 3.6 µm, the smaller ones being more numerous in the inner membrane. The fibres have a protein core surrounded by a mucopolysaccharide mantle (Tranter et al., 1983). The shell membrane protein contains the cross-linking amino acids desmosine and isodesmosine, and its structure and composition are quite unique and different from other fibrous proteins such as keratin, connectin, collagen or microfibrillar protein (Roberts and Brackpool, 1994). These fibres have a complement of antibacterial proteins (e.g. ovotransferrin, lysozyme, ovocalyxin) (Hincke et al., 2000; Gautron et al., 2001a; Gautron et al., 2001b; Gautron et al., 2011). The inner and outer shell membranes are attached at all points except at the blunt end of the eggshell where they separate and the air cell is formed.

9.3. The vitelline membrane

The vitelline membrane surrounding the yolk segregates the albumen and the yolk. This membrane is made up of three layers: the inner layer formed in the ovary; a continuous intermediate layer; and the outer layer deposited in the oviduct (Mann, 2008). The outer surface of the vitelline membrane in fresh eggs is composed of fibres connected to the chalaziferous layer. The proteins of the vitelline membrane are ovomucin, vitelline membrane outer layer protein I (VMOI) and lysozyme (about 60 % dry weight). Ovomucin appears to form the skeleton of the outer layer, but especially lysozyme is responsible for its integrity. Another protein isolated from the outer layer is the vitelline membrane outer layer protein II (VMOII) (Kido et al., 1992; Mann, 2008). The functions of VMOI and VMO II are unknown. The inner layer consists largely of the glycoproteins GPI, GPII and GPIII (Mann, 2008). The vitelline membrane also contains numerous enzymes whose functions are not entirely elucidated, including several ATPases and proteases (Mann, 2008).

9.4. The egg albumen

The egg albumen (also commonly known as the egg white) is a heterogeneous medium structured into four physically distinguishable zones, mainly depending on their ovomucin content and on the level of glycosylation of proteins. The chalaze layer, surrounded by the internal liquid egg albumen, is in contact with the yolk. It is surrounded by a sac of gelatinous albumen (thick albumen or albuminous sac) which is separated from the eggshell by a layer of more liquid albumen (outer liquid egg white), except at the ends of the egg. The albuminous sac, together with the chalazae, holds the yolk in a central position (Nys, 2010). The antimicrobial activity of egg albumen is well documented (see Baron et al. (2010) and Guyot et al. (2013) for reviews) and it serves as a protective barrier because of its viscosity, which impairs bacterial motility, its alkaline pH (around 9.3, attained within days of lay), and its nutritional deficiencies, especially available iron restriction. Egg albumen also contains an arsenal of antimicrobial molecules, including proteins (Table 3), peptides (Gong et al., 2010; Hervé-Grépinet et al., 2010) and antibodies. Lysozyme is a well-known inhibitory protein, expressing muramidase activity against gram-positive bacteria. A second non-specific and non-hydrolytic antimicrobial mechanism of lysozyme is thought to involve bacterial membrane disruption (Wang and Shelef, 1991; Ibrahim et al., 1996). The other well-known egg albumen protein exhibiting antimicrobial activity is ovotransferrin. The main activity of this protein involves chelation of metal ions, including iron, rendering egg albumen deficient in this essential element for bacterial growth. Other mechanisms of antimicrobial activity are also described for this protein, mainly involving membrane perturbation resulting from ionic interactions of its cationic form with bacterial membranes (Ibrahim et al., 2000; Aguilera et al., 2003).

Other proteins associated with the albumen include (i) vitamin sequesters with specific binding sites, e.g. avidin for biotin, riboflavin-binding protein for riboflavin and thiamin-binding protein for thiamin;

and (ii) inhibitors of proteinases (ovoinhibitor, ovostatin, cystatin, ovomucoid). Ovoinhibitor and ovomucoid are multi-domain Kazal-type inhibitors, with each domain containing an actual or putative reactive site for a serine proteinase. Cystatin is a cysteine proteinase inhibitor, while ovostatin inhibits proteinases from different classes (serine-, cysteinyl-, metallo-, and aspartyl-proteinases). Some minor components recently identified by high-throughput approaches might also play a role in the defence mechanisms of eggs against internal bacterial contamination. Examples of these are the ovalbumin-related protein X (OVAX) (Rehault-Godbert et al., 2013) and Tnp (analogous to the bactericidal permeability increasing protein-lipopolysaccharide binding protein family) (Guérin-Dubiard et al., 2006).

Additional factors that limit microbial growth are the presence of antibodies in the egg. Yolk contains IgY (IgG-like) while IgA and IgM are deposited in the egg albumen (Rose et al., 1974; Kovacs-Nolan and Mine, 2012; Bedrani et al., 2013). IgY, if present, occurs in very low concentration in egg albumen (Rose and Orleans, 1981).

This arsenal of antimicrobial molecules may interact in conjunction with other as yet unidentified egg albumen components. The level of expression of antimicrobial activity appears to be largely modulated by specific physicochemical parameters, such as a natural alkaline pH, temperature and the protective mechanisms lead to adverse ionic conditions within the egg that may lead to bacterial membrane perturbation and cell death. It is thought that bacteria deposited within forming eggs experience high levels of stress in egg albumen under the natural conditions the hen's genital tract (42 °C), and subsequently during hatching (around 42 °C and alkaline conditions). These protective mechanisms have evolved to safeguard the developing embryo within fertile eggs (see Alabdeh et al. (2011) and Baron and Jan (2011) for a review of this topic).

Table 3: Properties of the main antimicrobial proteins present in the albumen. Adapted from (De Reu, 2006)¹.

Protein	Antimicrobial activity
Ovotransferrin	Chelator of metal ions (particularly Fe ³⁺ , also Cu ³⁺ , Mn ²⁺ , Co ²⁺ , Cd ²⁺ , Zn ²⁺ and Ni ²⁺) Membrane perturbation
Lysozyme	(a) Cleavage of β(1–4) glycosidic bonds between <i>N</i> -acetylmuramic acid and <i>N</i> -acetylglucosamine in cell wall peptidoglycan (b) Membrane disruption (c) Formation of oligosaccharides from bacterial cell wall tetrasaccharides by transglycosylation (d) Flocculation of bacterial cells
Ovoflavoprotein	Chelator of riboflavin and other vitamins (G and B2)
Avidin	Chelator of biotin
Ovoinhibitor	Proteinase inhibitor
Ovomucoid	Trypsin and chymotrypsin inhibitor
Ovostatin	Proteinase inhibitor
Cystatin	Proteinase inhibitor
AvBD11 and gallin	Antibiotic peptides
OVAX	Proteinase inhibitor
Tnp	Binding protein

¹ Additional information was added from Chipman et al. (1968), Boekelheide et al. (1979), Davidson et al. (2005) and Guyot et al. (2013).

Concluding remarks

- Provided that the egg is fresh and undamaged, the cuticle, the eggshell, the shell membranes and the chalazae physically prevent bacterial penetration into the egg content.

- The vitelline membrane limits bacterial penetration into the most nutritious compartment of the egg, the egg yolk.
- The egg albumen, in particular, but also the cuticle, eggshell and shell membranes possess antibacterial properties.
- The antimicrobial activity of egg albumen is modulated by specific physicochemical parameters, such as a natural alkaline pH, temperature, and adverse ionic conditions that may lead to membrane perturbation in bacterial cells.

10. The effect of time and temperature on the quality of table eggs

The egg is at its highest quality when freshly laid, but quality declines over time, and particularly at high storage temperatures (Kirunda and McKee, 2000), leading to the deterioration of the physicochemical and structural integrity of its components, even under proper handling conditions. According to Stadelmann (1995), provided that the storage temperature is constant, a high relative storage humidity, i.e. in the range 75–80 %, is recommended for optimal conservation of the egg's structures and for the reduction of survival of *Salmonella* on the eggshell surface.

Under normal handling conditions, the decrease in egg quality occurs through the increase in the volume of the air chamber concomitant with the loss of egg weight. These changes are due to loss of water through the eggshell pores, liquefaction and an increase in the pH of the egg albumen, flattening of the egg yolk and a decrease of the strength of the vitelline membrane (Mertens et al., 2010). These modifications are mainly due to the gaseous exchanges between the egg's contents and the surrounding environment and also to the exchange of molecules such as iron or water between the egg albumen and egg yolk compartments. Temperature, time, and humidity are crucial parameters involved in these phenomena (Chen et al., 2005; Mertens et al., 2010). The quality of egg products is obviously dependent on egg quality.

10.1. Effect on cuticle and eggshell integrity

According to Bain et al. (2013), the intact cuticle is sufficient to avoid any bacterial penetration. By acting as a hydrophobic agent and a potential barrier, plugging the eggshell pores, it would be expected to play a major role in preventing microbial penetration, and when it is removed or damaged microbial contamination of the egg increases (De Reu et al., 2006c). The quality of the cuticle varies from egg to egg, which may influence the possibility for trans-shell contamination in poor hygienic conditions (Roberts et al., 2013). The cuticle progressively degrades over time, losing its defence capacity at a variable rate depending on factors such as humidity, egg temperature, ambient air temperature, and interventions such as improper egg washing (Vadehra et al., 1970; Ball et al., 1975; De Reu et al., 2006c). Protection by the cuticle was found to last at least up to four days at ambient temperature (Vadehra et al., 1970). It becomes brittle and begins to degrade about three weeks after the egg is laid (Viera, 1996).

The role of the cuticle as the first-line defence against *S. Enteritidis* has been questioned by Nascimento et al. (1992). Messens et al. (2007) also found that although the presence of the cuticle was important in lowering trans-shell penetration, its degree of deposition did not affect the occurrence of trans-shell penetration greatly. This study demonstrated that the cuticle deposition varies between batches of eggs.

Considering the eggshell strength, Aygun and Sert (2013) showed a decrease of around 10 % at both 5 °C and 22 °C during the first two weeks of storage. However, it then remained stable for up to 42 days at both temperatures. Jones and Musgrove (2005) demonstrated that the eggshell strength remains unchanged for 10 weeks at 4 °C. These results corroborate the assumptions of Vadehra et al. (1970) and De Reu et al. (2006b) on the absence of a relationship between eggshell quality and thickness and the level of bacterial trans-shell penetration. The essential factors affecting trans-shell penetration may include the level of contamination of the eggshell surface (Chen et al., 1996;

Miyamoto et al., 1998; Messens et al., 2006; Messens et al., 2007) and the presence of cracks or defects in eggshell calcification (Ernst et al., 1998; Allen and Griffiths, 2001).

It is, however, important to note that a positive temperature differential between the egg and the environment, due to the transfer of the warm egg into lower temperatures, creates a negative pressure within the egg due to contraction of the air cell (Messens et al., 2004). This pressure gradient, together with the presence of moisture on the eggshell surface, represents a risk of bacterial trans-shell penetration (Board and Fuller, 1994; Berrang et al., 1999) but without an obvious correlation with the level of contamination of the egg contents (De Reu et al., 2006b).

10.2. Effect on egg weight

Due to the loss of water vapour and carbon dioxide through the eggshell pores, the egg loses weight during storage. This phenomenon mainly concerns the egg albumen compartment. The level of gas loss depends on the porosity of the eggshell, the quality of the cuticle and environmental parameters, such as temperature, humidity and length of storage. Under stable humidity conditions, the weight loss increases with temperature. At a temperature around 10 °C and a relative humidity in the range 80-85 %, the kinetic of egg weight loss is sufficiently slow to allow long-term storage (Sauveur, 1988). After 28 and 42 days storage at 5 °C, the egg loses only 2.9 % and 3.8 % of its weight, respectively (Aygun and Sert, 2013). However, this process is greatly accelerated if the temperature increases and/or the relative humidity decreases: the percentage of egg weight loss is around three to four times higher at 25 °C than at 10 °C (Sauveur, 1988) or at 22 °C than at 5 °C (Aygun and Sert, 2013), other things being equal. According to Leleu et al. (2011), there is a strong influence of the length of storage on the percentage of egg weight loss at ambient temperature. A loss of around 5 % was observed at the end of a storage period of 5 weeks at ambient temperature.

As a consequence of egg weight loss, the increase in the height and in the volume of the air chamber may promote the breakage of the inner shell membrane, and/or lead to the separation of the inner and outer shell membranes, resulting in a 'mobile' air chamber. According to Leleu et al. (2011), the increase in the height of the air chamber is obvious from the first week of storage at ambient temperature. This phenomenon continues for up to five weeks, leading to a final three-fold increase in volume.

10.3. Effect on egg albumen pH and viscosity

Owing to the loss of carbon dioxide through the eggshell pores, the pH of egg albumen increases from about 7.6 to 8.9-9.4 after several days of storage at ambient temperature. The rate of pH increase is positively correlated with temperature. After two weeks of storage at 32 °C, the egg albumen pH is around 0.4 units higher than at 15 °C (Sauveur, 1988). The same difference was highlighted for storage at 5 °C vs. 22 °C, regardless of the duration of incubation (14, 24 and 42 days) (Aygun and Sert, 2013). The pH increase recorded by Leleu et al. (2011) at ambient temperature was lower, i.e. an increase of around 0.1 pH unit after the first two weeks of storage; the pH was unchanged for longer incubation periods, i.e. up to five weeks.

One of the main consequences of an increase in pH is the liquefaction of the thick albumen. Although this phenomenon has not been entirely elucidated, liquefaction is recognized as involving modifications of the structure of ovomucin and of the interaction of this with lysozyme (Sauveur, 1988; Mertens et al., 2010). Egg albumen liquefaction leads to a gradual migration of the yolk to the blunt end of the egg. This phenomenon is accentuated by the breakdown of the chalazae, enabling the egg yolk to come into contact with the shell membranes. The thinning of the thick albumen, most widely measured in Haugh units (Haugh, 1937), is faster at high storage temperature. While no change in Haugh units is detected after seven days incubation at 1 °C, a 12 % and 40 % decrease is observed at 15 °C and 32 °C, respectively (Sauveur, 1988). According to Aygun and Sert (2013), the decrease in Haugh units mainly occurs during the first two weeks of storage at 22 °C (41 % decrease), and a further decrease of around 15 % is observed from 24 to 42 days. These authors showed that this parameter was unaffected at 5 °C up to 42 days of storage. In the study of Leleu et al. (2011), a

decrease of around 16 % was observed after the first two weeks of storage at ambient temperature. Further incubation (up to five weeks) did not induce a further decrease.

10.4. Effect on egg yolk quality

Due to the osmotic pressure difference on either side of the vitelline membrane, and to the lower hydration level of egg yolk proteins, water moves from the albumen to the yolk during storage. This transfer is increased at high storage temperatures. The increase in egg yolk volume leads to the decrease of its viscosity, to its flattening and to the weakening of the vitelline membrane. These water transfers also lead to changes in the relative mineral contents of egg albumen and egg yolk. The transfer of mineral cations, such as calcium and magnesium, from egg albumen to egg yolk, could contribute to albumen liquefaction (Sauveur, 1988; Mertens et al., 2010). The yolk index, widely used to express the spherical nature and thus the quality of the yolk, decreases with time throughout the incubation period (up to 42 days) at 22 °C, while its value remains stable at 5 °C (Aygün and Sert, 2013). According to Leleu et al. (2011), the decrease in the yolk index was a function of time at ambient temperature. A final 15 % decrease was observed after five weeks incubation.

10.5. Effect on vitelline membrane integrity

According to (Guan et al., 2006), bacterial penetration through the vitelline membrane does not occur at 42 °C, the temperature of egg formation in the hen's reproductive tract. After laying, the strength of the vitelline membrane decreases during storage owing to the degradation of its structural integrity and to the increase in the egg yolk volume (Fromm, 1964; Back et al., 1982; Kirunda and McKee, 2000; Chen et al., 2005; Jones and Musgrove, 2005). If the membrane degrades, components of the yolk can diffuse into the albumen, resulting in a dramatic decrease of the antimicrobial defences of the egg albumen.

The strength of the vitelline membrane remains relatively stable at 4 °C for 6 to 10 weeks (Chen et al., 2005; Jones and Musgrove, 2005). However, its flexibility is reduced by half after a storage period of six weeks at 4 °C (Jones and Musgrove, 2005). At ambient temperature, its strength is quickly reduced. According to (Leleu et al., 2011), storage for five weeks at ambient temperature resulted in around a 20 % decrease in the strength of the vitelline membrane. According to Chen et al. (2005), after four weeks at 22 °C, it is no longer possible to carry out the physical measurement of its rupture-strength. During storage for one week at 18 °C, following seven months storage at 0 °C, an additional 16 % loss of its strength was recorded (Berardinelli et al., 2008).

To conclude, according to Chen et al. (2005), storage at 4 °C and perhaps 10 °C delayed the ageing process of eggs, preserving the antimicrobial activities of egg albumen and maintaining the egg structures. Low-temperature storage therefore has a significant impact on the safety and overall egg quality. The specific impact of the degradation of the vitelline membrane on the growth potential of pathogens such as *S. Enteritidis* is discussed in Section 11.2.

Conclusions

- The antimicrobial properties of egg albumen are maximal at the time of lay, but there is also an increase in egg albumen pH following the first days after laying, giving rise to alkaline conditions that further limit bacterial growth.
- Gaseous exchange between the egg contents and the atmosphere, as well as the exchange of molecules such as iron or water between egg albumen and egg yolk lead to a decrease in egg quality throughout storage.
- Temperature, time and humidity are crucial parameters involved in the reduction in egg quality throughout storage.
- The decrease in the protective action of the cuticle may begin from the fourth day of storage at ambient temperature.

- The strength of the eggshell decreases during the first two weeks of storage at ambient temperature and then remains stable for up to 42 days. This change appears to have no effect on bacterial trans-shell penetration, whatever the time and temperature of storage.
- Egg weight loss increases with time at ambient temperature and results in the movement of the yolk closer to the eggshell, potentially increasing the risk of bacterial penetration into the yolk.
- pH increases during the first two weeks of storage at ambient temperature leading to egg albumen liquefaction and also result in movement of the yolk closer to the eggshell.
- Increases in the volume of egg yolk at ambient temperature are associated with the gradual weakening and loss of flexibility of the vitelline membrane.
- Storage at chilled temperatures helps maintain egg quality, i.e. eggshell strength, egg weight, egg albumen pH, viscosity of the thick albumen and strength of the vitelline membrane.

11. Impact of storage conditions on pathogens and spoilage microorganisms

11.1. Effect of temperature and time on the survival of *Salmonella* on the eggshell

The survival of *Salmonella* on egg shells is possible, even in the absence of faecal material and is enhanced by low temperature. Survival is inversely related to both storage temperature and relative humidity (Humphrey, 1994; Messens et al., 2006). Studies on the association of storage temperature (between 15 °C and 25 °C) and relative humidity (between 45 % and 75 %) on the survival of *Salmonella* on eggshells showed that, at 25 °C, survival decreased when the relative humidity increased and vice versa (Messens et al., 2006).

On the other hand, a recent work performed by a research consortium (Anonymous, 2012) suggests that this relationship may not be linear; a deterministic risk model predicted 8 °C as the worst storage condition, corresponding to the lowest decrease in numbers of *Salmonella* on the eggshell. When an initial *Salmonella*-load of 3 log₁₀ CFU/g was considered in the model, both 4 °C and 21 °C were associated with undetectable levels of *Salmonella* after 28 days of storage, whereas 1.2 log₁₀ CFU/g was predicted on table eggs stored at 8 °C for the same period. The experiments were based on 10 repeats for each set of time–temperature conditions.

The penetration rate of *S. Enteritidis* is affected more substantially by the storage temperature than by the relative humidity; at 25 °C, the *S. Enteritidis* penetration rate was higher than at 20 °C and 15 °C, while no significant effect of relative humidity was observed (Messens et al., 2006). Moreover, the higher the initial load of shell contamination the higher the penetration rate.

During storage and transport, excessive temperature fluctuations at all stages until consumption should be avoided because this provokes water condensation and subsequent microbial growth. Water condensation on the eggshell is dependent on the surrounding temperature, relative humidity and eggshell temperature (Zeidler, 1994) and could be avoided by optimising the cooling process, storage and distribution of eggs. Generally, condensation or ‘sweating’ of eggs occurs when cold eggs are transferred to a higher temperature environment without adjusting the relative humidity, resulting in condensation of atmospheric moisture on the cold surfaces. Measuring the relative humidity in the new situation and adjusting the temperature and/or relative humidity may help avoid condensation.

Similarly, a previous EFSA Opinion (2009b) concluded that “*the relationship between environmental temperature, relative humidity and actual egg shell temperature affects the development of condensation. Cold chain disruption is one factor increasing the risk of condensation and this could increase bacterial penetration into the egg. There is evidence indicating that cross-contamination of egg shells can occur at the processing level. The probability of this cross-contamination depends on the proportion of Salmonella-contaminated eggs, technology and hygienic practices*”. The Opinion also stated that there are not sufficient data to evaluate the occurrence of trans-shell penetration and growth of *Salmonella* due to cross-contamination during processing.

11.2. Effect of temperature and time on the growth of *Salmonella* in eggs contents

As explained above (Section 8.1), deposition of *S. Enteritidis* directly into egg contents is thought to occur primarily in the albumen, typically at a site near the vitelline membrane. Both the site of deposition and the storage temperature will influence the growth of *Salmonella* in eggs. This has been documented in detail in the previous scientific opinion on cooling of table eggs (EFSA, 2009b) and in the review paper by Gantois et al. (2009).

In egg albumen, *S. Enteritidis* grows slowly and only to a limited extent because the albumen contains multiple antimicrobial components, inducing bacterial cell wall and DNA damage (Gantois et al., 2009). At less than 8 °C, *Salmonella* is unable to grow in albumen (Ruzickova, 1994; Schoeni et al., 1995) and a bactericidal effect is observed at 4 °C in a few days (Lock and Board, 1992; Schoeni et al., 1995; Chen et al., 2005). The same observation at 10 °C was made by Clay and Board (1991) and Chen et al. (2005), while Schoeni et al. (1995) observed a weak growth in egg albumen. In egg yolk, the growth is weak at 10 °C (Gast and Holt, 2000; Gumudavelli et al., 2007) and no growth is observed at 7 °C (Gumudavelli et al., 2007). Between 20 °C and 30 °C, the growth of *Salmonella* is possible in all egg components, with higher growth rates in egg yolk, where the population can increase by 7 to 8 log units in less than two days (Ruzickova, 1994; Schoeni et al., 1995; Gast and Holt, 2000, 2001; Gumudavelli et al., 2007). In egg albumen, the growth rate is lower, the bacterial population increasing from 1 to 4 log units, depending on the temperature and the incubation time (Clay and Board, 1991; Humphrey and Whitehead, 1993; Schoeni et al., 1995; Cogan et al., 2001; Dubocage et al., 2001; Gast and Holt, 2001; Chen et al., 2005; Messens et al., 2005; Murase et al., 2005). Humphrey and Whitehead (1993) and Gast and Holt (2000) observed that in artificially contaminated eggs, there is about 1 log of growth of *S. Enteritidis* during the first 24 hours post-inoculation, probably as a result of the availability of bacterial intrinsic iron reserves. This may however also be an experimental artefact due to culture medium being transferred with the bacterial inoculum and contaminated forming eggs have been shown to have a higher contamination rate than eggs after lay due to the bactericidal effect of the albumen at the body temperature of the hen ((Keller et al., 1995). After lay, cells enter a lag phase, where, in the majority of eggs, there is little or no change in the *Salmonella* counts until the yolk membrane begins to deteriorate, releasing nutrient into the albumen.

When *Salmonella* is inoculated into the albumen of whole eggs, growth is faster than in separated albumen. In addition, high *Salmonella* counts were found in the yolk, indicating migration towards the yolk (Cogan et al., 2001; Messens et al., 2004). The integrity of the vitelline membrane becomes lost during storage, resulting in leakage of nutrients into the albumen. This is considered to allow the bacteria to migrate to the vitelline membrane and multiply and invade the nutrient-rich egg yolk (Humphrey and Whitehead, 1993). It is believed that the vitelline membrane in table eggs inhibits yolk invasion by *Salmonella* as there is a delay before yolk penetration and fast growth in the yolk. This was observed upon storage at room temperature of contaminated eggs from both naturally (Humphrey and Whitehead, 1992) and artificially (Gast and Beard, 1992) infected hens. Leleu (2011) demonstrated that the yolk membrane of 20 %, 54 % and 84 % of eggs can be penetrated after 4, 7 and 14 days of storage at 20 °C, respectively. In this study, which must be considered to represent a worst case scenario, intact egg yolks of fresh eggs were removed from the egg, and the external surface of the yolk membrane was inoculated with approximately 412 cells of *S. Enteritidis*, overlaid with pasteurized albumen and stored at 20 °C for 21 days. During storage, yolk contents were withdrawn and assessed for the presence of *Salmonella*. Gantois et al. (2008) observed penetration of the yolk membrane in 57 % of eggs after four days at 25 °C using a similar approach based on inoculation with approximately 100 cells of *S. Enteritidis*.

Higher temperatures and longer storage times generally favour loss of membrane integrity (see Section 10.5 for additional details) and can result in *S. Enteritidis* crossing the vitelline membrane before the egg is consumed. Whiting et al. (2000) analysed the data presented in Humphrey and Whitehead (1993). In that study, table eggs (collected within 2 hours post lay) were stored at 8–37 °C and at time intervals of three days, 10 eggs were removed. These eggs were aseptically broken and the albumen

next to the yolk was inoculated with circa 500 cells of *S. Enteritidis* to determine when the albumen supported growth of *S. Enteritidis* and thus when the yolk membrane disintegrated (this is referred to as the Yolk Membrane Breakdown Time or YMT). The latter was (arbitrarily) defined when 20 % (2 out of 10) of eggs sampled at a certain storage time had *S. Enteritidis* counts greater than 10^4 CFU per egg after four days of storage at 20 °C. Results are shown in Table 4.

Table 4: Storage times (days) at various temperatures before the egg albumen will allow *S. Enteritidis* (SE) growth (adapted from Whiting et al. (2000))

Egg storage temperature (°C)	Storage time before the egg albumen will allow SE growth ^(a)	Egg storage temperature (°C)	Storage time before the egg albumen will allow SE growth ^(a)
12	28; 28; 28; 35; 42; 42	25	6; 12
16	21; 28; 28; 28	27	6; 6; 7; 12
20	14; 17; 27; 28; 28; 42	30	6; 6; 7; 12
23	18	37	3
24	7; 7; 10; 10; 12		

(a): the different figures represent replicate trials.

In order to model bacterial growth as a function of temperature, there are several models available in the scientific literature (Hinshelwood, 1946; Ratkowsky et al., 1982; Zwietering et al., 1991; Baranyi et al., 1993; Rosso et al., 1993; Whiting et al., 2000). They are based on either a mechanistic or empirical approach. They can all be considered as valid to describe the effect of temperature on the specific growth rate during the exponential phase, although there can be differences in the confidence limits for the parameters estimated (Rosso et al., 1993). The lag phase is (i) not considered (Hinshelwood, 1946; Ratkowsky et al., 1982; Rosso et al., 1993), (ii) assumed to be negligible, (iii) subsumed in time to yolk membrane breakdown above minimum growth temperature (Paoli, 2001) or (iv) estimated as a two-parameter calculation or two-phase linear growth model. Different models have been used successfully in published studies with different transformations (e.g. log transformation of the Rosso model in the AECL model (Thomas et al., 2006)).

11.3. Effects of temperature and time on pathogenic microorganisms other than *Salmonella*

The ability of *C. jejuni* to penetrate and colonise eggs after artificial shell contamination *in vitro* was investigated (Allen and Griffiths, 2001). The eggs were colonised after 24 hours' incubation at 37 °C, 40 °C, and 42 °C under microaerophilic conditions. The extent to which colonisation occurred varied with temperature. The most extensive colonization was observed at 42 °C, followed by 37 °C and 40 °C. Unlike microaerophilic conditions, under aerobic conditions, the eggs were colonized at similar levels for all three temperatures, showing that temperature had less of an effect on levels of contamination under these conditions. However, Sahin et al. (2003) suggest that survival of *C. jejuni* is probably a rare event, since the bacteria was not detected from any of 80 pools of 10 eggs stored at 18 °C for seven days after inoculation of the bacteria into the egg yolk.

Brackett and Beuchat (1992) showed that both low (2 log₁₀ CFU/egg) and high (4 log₁₀ CFU/egg) initial levels of *L. monocytogenes* on the eggshell surface decreased to less than 10 CFU/egg after six days of storage at 5 °C and 20 °C. After six weeks' storage, the bacteria were unquantifiable at both temperatures, even though they were still detectable by enrichment.

To our knowledge, the *B. cereus* group has never been assessed for its ability to penetrate the egg, and even less so regarding the effect of time and temperature on this ability. However, this event, as well as subsequent egg invasion, may be considered to be negligible, as the gram-positive flora show poor viability in egg albumen (see Section 9.4). Moreover, under proper flock management practices, their concentration on the eggshell surface is low just after laying (Koné et al., 2013). As described in the next Section (11.4), these bacteria are more likely to be involved in spoilage events in the context of egg product processing (Baron et al., 2007; Jan et al., 2011; Koné et al., 2013; Techer et al., 2014).

The impact of a prolongation of the storage time on the risk posed by these pathogens is unclear but it is likely to be small, particularly due to the decline of the organisms on the shells associated with longer storage time. The contribution of eggs and egg products to human illnesses caused by these pathogens is likely to remain minor in comparison with other foodborne and non-foodborne sources.

11.4. Effects of temperature and time on spoilage bacteria

The spoilage of eggs is related to eggshell contamination and also to the ability of specific bacteria to penetrate the egg, to evade and overcome the antimicrobial activities of egg albumen, and to metabolize the complex nitrogen and carbon sources of the egg contents, thus rendering eggs suitable for supporting growth and expression of various enzymatic activities and leading to spoilage events.

Poor egg production practices leading to eggs coming into contact with material heavily contaminated with spoilage bacteria, including faeces, litter, hay, straw and sawdust, poor egg handling practices and the presence of cracks or defects in eggshells are recognized as increasing the risk of spoilage issues (Dockstader, 1952; Harry, 1963; Ernst et al., 1998; Allen and Griffiths, 2001). However, if the eggs are clean and appropriate handling practices are maintained, including controlled storage temperatures, the egg should have a longer shelf life.

The literature on shell egg spoilage is often quite dated and rather descriptive. According to Haines (1938), the number of rotten eggs in commercially stored eggs is not generally more than 5 % and often less. The improvements made in modern egg production and distribution should have significantly reduced the cell numbers of aerobic bacteria, including Enterobacteriaceae, *E. coli*, yeasts and moulds, according to Musgrove et al. (2005b).

In order to evaluate the effect of time and temperature on egg spoilage issues, the level and type of microorganisms present on the eggshell surface and their ability for trans-shell penetration and for metabolising egg substrates should be considered. The period and conditions of exposure and the type and initial number of spoilage bacteria on the eggshell surface may influence bacterial trans-shell penetration rate, egg invasion and subsequent spoilage.

The level of bacteria present on the eggshell surface ranges between 3.8 and 6.3 log CFU/egg depending on the study, with an average value of 4.5 log CFU/egg (Moats, 1981; Lucore et al., 1997; Favier et al., 2000; Jones et al., 2004; De Reu et al., 2005; Musgrove et al., 2005a; De Reu et al., 2006b). The gram-positive flora is dominant at the surface, comprising the genera *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Aerococcus* and, to a lesser extent, *Bacillus* (Moats, 1979; De Reu et al., 2006b). Other less common contaminants are gram-negative bacteria of the genera *Salmonella*, *Escherichia*, *Alcaligenes* and *Pseudomonas* (Mayes and Takeballi, 1983; Board, 1994). According to Mayes and Takeballi (1983), Board and Tranter (1995) and Protais et al. (2003), eggshell contamination could arise from dust, soil or faeces and the presence of bacteria could be ascribed to their tolerance to dry conditions. (Gast et al., 1998) also identified airborne transmission as the main route of contamination in the poultry house environment.

The ability to penetrate eggshells is recognized as being strain dependent. According to Board (1994), while less prevalent at the egg surface, gram-negative bacteria are recognized as better at surviving the natural egg mechanical and physicochemical defences, facilitating their access to the internal egg compartments. Moreover, the internal properties of the egg favour growth of gram-negative bacteria, which have relatively simple nutritional requirements and the ability to grow at low temperatures (Board, 1977). Motility is another parameter facilitating penetration of the egg contents (Harry, 1963). This ability allows bacteria to move towards an attractor or away from a repellent (Grijspeerdt, 2001). *E. coli* and *Pseudomonas* are motile gram-negative bacteria that can more easily penetrate the eggshells than can non-motile bacteria such as *Staphylococcus*, which are passively distributed by contact or surface moisture (Grijspeerdt, 2001).

Concerning invasion of the egg contents, spoiled eggs have been described as containing a mixed contamination of gram-negative and a few gram-positive bacteria (Colmer, 1948; Dockstader, 1952;

Florian and Trussell, 1957; Mayes and Takeballi, 1983; Board and Tranter, 1995; Board, 2000; De Reu, 2006; De Reu et al., 2008). In terms of occurrence in naturally contaminated eggs, the most common bacteria described are *Pseudomonas fluorescens* and *P. putida*, *Proteus* and *Alcaligenes*. *Flavobacterium* spp., *Cytophaga* spp., *Aeromonas* and *Serratia* are uncommon and *Bacillus*, *Arthrobacter*, *Micrococcus*, and *Streptococcus* species were isolated occasionally (Board, 2000). The bacteria listed above have been associated with eggs produced in Australia, South Africa, Canada and the United Kingdom (Board, 2000). There appears to have been no change in their incidence over a 30-year period, corroborating the assumptions that the type of the spoilage flora is more relevant to intrinsic egg properties than to the geographic location, the period of collection and the conditions of egg production (Board, 2000).

(Florian and Trussell, 1957) classified spoilage bacteria as primary and secondary egg invaders. The species they considered as primary invaders were of the genera *Pseudomonas*, *Alcaligenes*, *Proteus*, and *Flavobacterium*. The secondary invaders comprised the genera *Achromobacter*, *Alcaligenes*, *Escherichia*, and *Flavobacterium*. All these microorganisms are gram-negative rod-shaped bacteria. In more recent studies, *P. aeruginosa* was also considered as more frequently invading the egg than other spoilage bacteria such as *S. aureus*, *E. coli*, *Serratia*, and *Acinetobacter* (De Reu et al., 2006c; Al-Bahry et al., 2012). *Pseudomonas* and *Alcaligenes* were also considered as more invasive than *S. Enteritidis* (De Reu et al., 2006c).

The spoilage characteristics of ‘rotten’ eggs, and the bacteria associated with these events are described as follows (Harry, 1963; Hayes, 1995; Board, 2000):

- fluorescent green rot due to *P. putida*;
- black rot (H₂S or putrid odour) due to *Pseudomonas*, *Proteus*, *Aeromonas*, *Alcaligenes*, *Escherichia* and *Enterobacter* spp.;
- blue rot due to *P. aeruginosa*;
- pink rot (after green rot) due to *P. fluorescens*;
- red rot (no odour) due to *Serratia marcescens* and *Pseudomonas* spp.;
- green rot (almond-like odour) due to *Stenotrophomonas maltophilia*;
- creamy colour of the yolk and tan colour of the albumen due to the *B. cereus* group;
- colourless and fruit odour due to *Acinetobacter*, *Moraxella* spp. and *Citrobacter*;
- colourless and mustiness, watery whites due to *Alcaligenes* and *Achromobacter*.

A yellow pigmentation of the shell membrane is also described. This type of spoilage involves the genera *Flavobacterium* spp. and *Cytophaga* spp.

On some occasions, moulds of the genera *Penicilium*, *Alternaria*, and *Mucor* can spoil eggs and produce different types of fungal rots (Silliker, 1980).

Considering the effect of time and temperature on the development of rots, it is generally recognized that the longer the exposure of eggs to bacteria and the greater the contamination of eggshells, the higher the penetration rate (Javed et al., 1994; Chen et al., 1996; Miyamoto et al., 1998; Messens et al., 2006; Messens et al., 2007; Al-Bahry et al., 2012). The level of eggshell contamination is also recognized as depending on the season: lower counts are observed in winter, corresponding to low environmental temperatures, for the total aerobic flora and gram-negative bacteria (De Reu et al., 2005), for the total aerobic bacteria and *Enterococcus* (Mallet et al., 2006) and for the *B. cereus* group (Koné et al., 2013). Quarles et al. (1970) also suggested that high temperatures might lead to higher levels of eggshell contaminants. However, the level of eggshell contamination by total aerobic flora and gram-negative bacteria was shown to decrease over a storage period of nine days or more in both non-refrigerated and refrigerated eggs (De Reu et al., 2008).

According to Board (1994, 2000), a lag period of 7–20 days at ambient temperature is needed between contamination of the shells of newly laid eggs and the occurrence of microorganisms in the egg contents. The incidence of bacterial penetration increases with elevated holding temperatures. Studies at 9, 25 and 35 °C showed maximum bacterial activity at 25 °C (Board, 1994). Even if they are more prevalent on the eggshell surface, gram-positive bacteria are rarely identified in rotten eggs during storage at ambient or refrigerated temperatures (Board, 2000). However, the gram-positive bacteria may induce egg spoilage at higher storage temperatures, i.e. in the range 37 to 39 °C (Board, 2000). When eggs are held at a temperature of 15 to 30 °C, there is a lag of 10 to 20 days before the recovery of appreciable numbers of viable microorganisms and before the development of macroscopic changes in the egg contents. Invading bacteria may be confined to the shell membranes during this period until the egg yolk makes contact with the membranes. After this first step in which bacterial multiplication is confined to eggshell membranes, a second phase of multiplication leads to bacterial invasion of the egg contents and the first signs of spoilage appears when chromogenic and hydrolytic bacteria are involved (Board and Ayres, 1965). However, there was no evidence of any union between the yolk and the shell membranes for eggs artificially contaminated with *P. fluorescens* and stored at 10 °C (Board and Ayres, 1965). For these eggs, an early and persistent contamination of the egg albumen compartment was observed. According to these authors, the temperature influences the pattern of microbial invasion in three ways: (i) rate of bacterial multiplication during the phase in which this is confined to the shell membranes; (ii) the level of the antimicrobial activity of egg albumen; and (iii) the onset of the secondary phase of bacterial multiplication by virtue of its effect on the rate of deterioration of the internal egg quality. There is hence a clear relationship between the deleterious effect of high storage temperatures, the degradation of the internal quality of egg albumen and the rate of development of macroscopic changes in eggs infected with spoilage bacteria.

Considering the time of appearance of rots due to *Pseudomonas*, Elliott (1954) showed that aged or stored eggs, held at 15 °C and 87 % relative humidity for 58 days, became contaminated more rapidly than fresh eggs after artificial contamination by immersion in a suspension of *Pseudomonas*. When inoculated on the inner shell membrane, colonies appeared without penetrating into the egg albumen in the first days of storage. Black-light fluorescent pigments were shown to diffuse into the egg albumen to form bacteria-free fluorescent spots in one or two days. The time required for the bacteria to penetrate the inner membrane varied from one to more than eight days. The spread of fluorescent pigment from the site of primary infection throughout the egg took 2 to 11 days at 15 °C in eggs inoculated in the air cell. During this process the bacterial count increased to 7 CFU/g, corresponding to the fluorescence of the whole egg albumen compartment and appearance of distinct odours of decomposition. Further storage resulted in counts of 8 to 9 log CFU/g.

In a study in which eggshell bacteria were collected and their spoilage potential assessed *in vitro* (Harry, 1963), the pigment-producing pseudomonads likely to produce egg spoilage were obviously of importance. Their incidence was also shown to be particularly high in hay, straw and faeces. All 25 eggs taken from a deep litter were found to be contaminated with *Pseudomonas* species associated with the green rot of eggs, the average number on eggshells being around 4 log CFU/eggshell. These bacteria were detected in only one of 25 eggs collected in a battery unit. In the *in vitro* tests, these bacteria were able to coagulate egg contents, some only at 27 °C, some only at 37 °C and some at both temperatures, but the fluorescence pigment in all pigment-producing strains isolated was produced only at 27 °C. A relatively higher level of non-pigment-producing pseudomonads and bacteria of the *Alcaligenes* and *Achromobacter* species were highlighted in eggs contaminated with faeces, hay and straw. These species were shown to have capabilities for egg spoilage similar to the ones of the pigment-producing pseudomonads. The spoilage characteristics were, however, mustiness and watery egg white, without the appearance of discoloration.

Concluding remarks

- Survival of *Salmonella* on the shell of eggs is inversely related to both storage temperature and relative humidity, whereas the penetration rate of *S. Enteritidis* is more substantially affected by the storage temperature than by the relative humidity; at 25 °C, *S. Enteritidis* penetration

rate is higher than at 20 °C and 15 °C, while no significant effect of relative humidity is observed. Moreover, the higher the initial load of shell contamination the higher the penetration rate.

- The growth of *S. Enteritidis* within the egg is positively influenced by the storage temperature and is higher in the yolk than in the albumen. Higher temperatures and longer storage times generally favour loss of membrane integrity and can result in *S. Enteritidis* crossing the vitelline membrane before the egg is consumed.
- Survival of *C. jejuni* in eggs is unlikely, as the bacteria was not detected from any of 80 pools of 10 eggs stored at 18 °C for seven days after inoculation of the bacteria into the egg yolk.
- *L. monocytogenes* on the eggshell surface decreases rapidly during storage, remaining detectable by enrichment after six weeks' storage but not quantifiable.
- *B. cereus* group has not been assessed for its ability to penetrate the egg, nor regarding the effect of time and temperature on this ability. However, this event, as well as subsequent egg invasion, may be considered to be negligible, since gram-positive flora show poor viability in egg albumen.
- The impact of a prolongation of the storage time on the risk posed by pathogens other than *Salmonella* is unclear, but nevertheless the contribution of eggs and egg products to human illnesses caused by these pathogens is likely to remain minor in comparison with other foodborne and non-foodborne sources.
- Bacterial spoilage issues strongly depend on the hygienic conditions of egg production and practices of egg handling, including storage times and temperatures.
- Gram-positive genera such as *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Aerococcus* and, to a lesser extent, *Bacillus* dominate on the shell surface. Gram-negative genera, such as *Escherichia*, *Alcaligenes*, *Proteus*, *Flavobacterium* and *Pseudomonas*, are minor surface contaminants but have a greater ability to penetrate eggs, resulting in contamination and, eventually, spoilage. On some occasions, moulds of the genera *Penicilium*, *Alternaria*, and *Mucor* can also spoil eggs.
- The level of eggshell contamination by total aerobic flora and gram-negative bacteria was shown to decrease over a storage period of nine days or more, both in non-refrigerated and refrigerated eggs.
- At ambient temperature, there is a lag period of around 10 to 20 days before detection of appreciable numbers of trans-shell penetrated spoilage bacteria in the egg contents and before the appearance of macroscopic changes in the egg contents.
- Spoilage of table eggs is mainly the result of macroscopic changes in their odour and/or colour or viscosity, which would prevent the egg being used for food products.
- There appears to be a clear relationship between the deleterious effect of high storage temperatures, the degradation of the internal quality of egg albumen and the rate of development of macroscopic changes in table eggs infected with spoilage bacteria. While the effect of the storage temperature on the level of surface bacteria is variable according to a combination of conditions, an increase in temperature and/or in time has a negative impact on the egg defence mechanisms, increasing the risk of multiplication of the spoilage flora inside the egg contents.
- The relationship between environmental temperature, relative humidity and eggshell temperature affects the development of condensation. Cold chain disruption is one factor increasing the risk of condensation and this could increase bacterial penetration into the egg.

12. Freshness/quality criteria of eggs and egg products

The microbial and physicochemical qualities of egg products depend on the freshness and quality of the raw material. The conditions under which the eggs are stored and particularly the conditions of temperature and humidity, as well as the length of the storage period, may have dramatic consequences on the physicochemical and microbial qualities of raw egg products. Moreover, the decrease in the physicochemical properties of the egg contents may impact directly on the behaviour of bacteria in egg products. The main parameters that are susceptible to change during egg storage (see Section 10), and which may have an impact on the quality of the final egg products, include:

- the weakening of the integrity of the cuticle and of the eggshell, and particularly if the bacterial load of the eggshell surface is high, if moisture is also present and if there is an abrupt positive temperature differential during egg storage;
- the loss of egg weight, linked to the increase in the volume of the air chamber, the separation of the eggshell membranes and the breakage of the inner shell membrane, giving rise to a moving air chamber;
- the exchange of water and minerals between the egg yolk and egg albumen compartments, leading to the decrease in egg yolk viscosity and in the strength of the vitelline membrane;
- the increase in egg albumen pH leading to a decrease in its viscosity.

These physicochemical changes may have several damaging consequences on the quality of the egg products, as follows:

- enhancement of the probability of bacterial penetration upstream of the breaking step and of the risk of cross-contamination at the step of egg breaking;
- decrease in the segregation between the egg white and the egg yolk compartments, with a damaging impact on the physicochemical properties of egg white and egg yolk, particularly their viscosity, which influences both the functional qualities of the egg products and the subsequent bacterial growth.

Concerning specifically egg white products, storage has a beneficial effect on control of bacterial development by increasing the egg white pH up to around 9.3 for several days after laying, there is a negative impact on the viscosity and on microbial quality if traces of egg yolk have been introduced by poor separation between the egg compartments.

In Appendix A, the effect of egg storage on egg product quality is further analysed in regard to physicochemical criteria and to subsequent microbial issues.

12.1. Evaluating the quality of table eggs

12.1.1. Evaluating the quality of the physical barriers against invading bacteria

The eggshell acts as a natural packing material for the egg contents, preventing the penetration of harmful bacteria (see Section 9). The quality of the cuticle, the shell strength, the absence of eggshell cracks, and the antimicrobial activity of albumen are prerequisites for achieving egg product quality (see Section 9.4). During storage on farms or in the packing centres, as well as during transport, poor handling practices can lead to the disruption of the egg's physical barriers. The presence of cracks increases the risk of bacterial contamination of the broken egg and of other eggs if the cracked ones leak, affecting the quality of the shell and that of the egg contents.

The development of sensors is a key step towards optimizing egg quality. Different indicators are described that are evaluated by fast, reliable and automated technologies. The choice of techniques depends on the consistency within a single product, the measurement speed, the cost of the instrumentation and the sorting efficiency required (see Mertens et al. (2011) for a review).

The quality of the cuticle can be controlled by differential quantification of the colour or the egg before and after staining (Messens et al., 2005; De Reu et al., 2006b; De Reu et al., 2006c; Leleu et al., 2011).

There are different traditional techniques for the assessment of the mechanical properties of the eggshell, mainly determined by its strength and the presence of cracks:

- direct methods: measure the compression fracture force, puncture tests and impact tests.
- indirect methods: measure the shell thickness, egg density and deformation of the egg during a non-destructive compression test. Novel dynamic methods have been developed, such as the acoustic/vibration test response, characterising the eggshell strength through the resonant frequency of an egg after it has been subject to an impact.

The detection of egg cracks is mainly carried out by egg candling. In the conditioning centres, this technology represents the key step for ensuring the microbial quality of the eggs. However, candling is not a very effective way of identifying dirty eggs when they are not washed. Egg handling companies have therefore invested in new technologies for automation of candling. The automated systems are mainly based on camera surveillance. This technique allows the detection of cracks and also quantification of dirt stains caused by contaminants such as faeces, uric acid, yolk, albumen and blood. An automated system based on vibration analysis also provides a reliable method for detecting cracks and defects in eggshells, including micro-cracks.

Shell membranes also act as physical and chemical barriers to potentially invading bacteria (see Section 9.2). Evaluating their quality is therefore essential. The risk of structural variation in these membranes also increases with time. Two parameters should be considered: the adhesion force between the external membrane and the shell and the resistance to breakage, both determined by mechanical destructive tests. The analysis of the air chamber should also give information on egg freshness and quality. A freshly laid egg has a small air cell located at the blunt end of the egg. The observation of the position of the air chamber by egg candling therefore allows the identification of damage in the inner shell membrane. Abnormal air cells are also indicators of weakening of the adhesion of the inner and outer shell membranes. Acoustic tests also highlight abnormality in the air cell, inducing asymmetry in the vibration response of the egg.

12.1.2. Evaluating the internal quality of the egg

From a microbiological point of view, two parameters should be considered to assess the internal egg quality: quality of the albumen and of the vitelline membrane (see Sections 9 and 10).

12.1.3. Quality of the albumen

The consistency and clarity of albumen are described as being key parameters of egg freshness. The quality of the albumen is usually expressed in terms of height of air cell or height of the thick albumen or in terms of Haugh units. The last parameter is a reliable indicator of egg freshness; the better the quality and freshness of the egg, the higher the Haugh units. Other methods have been developed for predicting egg freshness or albumen quality, but they remain at the experimental scale and further research is needed before they can be used in in-line sorting machines. Such methods are based on spectral techniques (Visible/Near-Infrared Spectroscopy, Proton Nuclear Magnetic Resonance and front-phase fluorescence spectroscopy), dielectric properties and the recording of chemical volatiles by an 'electronic nose'.

12.1.4. Quality of the vitelline membrane

The physical quality of the egg yolk can be evaluated by an index defined as the ratio of the yolk height to its width. This parameter is a relevant indicator of the quality of the vitelline membrane surrounding the yolk. A fresh egg typically exhibits a yolk index of around 0.45. As the egg ages, the transfer of water from the albumen to the yolk increases its size, leading to the weakening of the

vitelline membrane. The strength of the vitelline membrane can be considered a measure of egg freshness. During egg-breaking processes, the vitelline membrane has to remain intact for an efficient separation of egg white and yolk. Its strength is then a crucial parameter for improving the quality of the egg products. Capillary vacuum techniques are used for assessing the strength of the vitelline membrane. More recently, other methods have been developed, which measure the rupture energy and the maximum force, obtained by driving a probe into the highest point of the yolk, by means of a compression (texture) device.

12.2. Evaluating the microbiological quality of egg products

In the following paragraphs, several methods published in the literature for the assessment of the microbial quality of egg products are described, including the ones devoted to the search for the regulation criteria and also to spoilage microorganisms.

Classic microbiological methods or alternative ones are described for the evaluation of freshness and microbial quality of various egg products (see Table 5). These methods are intended to target either relevant bacteria or metabolites considered as relevant metabolic markers of spoilage, including the organic acids described in the regulation criteria.

Table 5: Listing of the available alternative methods for the assessment of the microbial quality of liquid egg products (see the text for references)

Methods	Liquid egg product/egg-based food tested	Spoilage criteria	Advantages	Drawbacks
Targeting spoilage bacteria				
Polymerase chain reaction tests	Whole egg and egg-containing foods	Detection and identification of spore-forming bacteria	Sensitive Fast Reliable	Only intended for spore-forming bacteria No discrimination between spoiling from non-spoiling strains
Isothermal calorimeter	Whole egg	Microbial growth, enzymatic activities	Sensitive	Equipment difficult to use
Optical system based on red light	Egg white	Bacterial growth	Suitable for developing predictive models	Requirement for high bacterial counts ($>10^6$ CFU/g)
Targeting spoilage markers				
Odour analysis	Whole egg, egg white and egg yolk	Slightly sour, putrid odours	Sensitive Fast	Requirement for qualified panels Expensive
Gas chromatography–Mass Spectrometry	Whole egg, egg white and egg yolk	Dimethylsulphide	Sensitive	High technical constraints
High-performance liquid chromatography	Whole egg	Uracil, lactic and acetic acids	Fast	Not sensitive enough
Enzymatic kits	Whole egg and fresh pasta	3-Hydroxybutyric, succinic and lactic acids, uracil	Fast	Low extraction yield Not sensitive

12.2.1. Classic microbiological methods

There are classic microbiological methods that may be used by the manufacturer to detect the psychrotrophic flora and/or the spoilage spore-forming bacteria such as the *B. cereus* group. However, these methods are time consuming, particularly when psychrotrophic strains are targeted. A method based on quantitative PCR has been developed, allowing specific identification of spore-forming bacteria in whole egg (Postollec et al., 2010). As evaluated in samples of contaminated egg products,

this method offers the advantage of identifying species that are not detected by the standard microbiological method. It also renders possible the identification of and discrimination between the different *B. cereus* group members, including the psychrotrophic *B. weihenstephanensis* species. Nevertheless, discrimination between spoiling and non-spoiling strains remains unfeasible.

12.2.2. Effect on the concentration of the organic acids relevant to the regulation criteria

The regulation describes specific requirements for the manufacture of egg products. Considering the microbiological criteria, Regulation (EC) No 2073/2005, defines (i) a food safety criterion for *Salmonella* in 'egg products, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk' for egg products placed on the market during their shelf life (absence in 25 g, as evaluated by the reference EN/ISO 6579 method), and (ii) a process hygiene criterion for Enterobacteriaceae at the end of the process of egg product manufacturing (less than 100 CFU/g, as counted by the standard ISO 21528-2 method).

Freshness criteria are defined in Regulation (EC) No 853/2004, as follows: (i) the concentration of 3-hydroxybutyric acid must not exceed 10 mg/kg in the dry matter of the unmodified egg product; and (ii) the lactic acid content of the non-fermented raw material (i.e. where any lactic acid bacteria has been added for de-sugaring purposes) used to manufacture egg products must not exceed 1 g/kg of dry matter.

There are very few data on these quality indicators, which prevents a quantitative assessment of the evolution of the concentration of these indicators with storage time.

Of the two currently recommended indicators, 3-hydroxybutyric acid is exclusively related to the use of embryonated eggs (Alamprese et al., 2004), and so it is related to fraud more than to microbial growth or the conditions of storage, since its concentration is not influenced by storage time if eggs are not embryonated. Even if present at trace levels in infertile eggs, its concentration does not increase during storage, regardless of the storage conditions.

Lactic acid is recognized as an indicator of microbial degradation of table eggs. It is present in the egg as a result of both the development of the embryo in fertile eggs and microbial growth (Stijve and Diserens, 1987). Its level will be affected by the conditions of storage—its concentration increasing with egg storage time. In shell eggs, lactic acid is present at a concentration that increases with storage time.

Lactic acid in table eggs is present at an average concentration of 250 mg/kg (Carazzolo et al., 2002). Hidalgo et al. (2008) detected lower levels, i.e. as low as 1 to 7 mg/kg, in eggs 10 days after laying. In the study by Hidalgo et al. (2004), the level of lactic acid was also below the legal limit for grade A-extra retail eggs. In the study by Rossi et al. (2010), no detectable lactic acid was found in newly laid eggs obtained directly from a farm or in grade A-extra eggs from a commercial channel.

According to Carazzolo et al. (2002), the concentration of lactic acid has a tendency to increase throughout the storage period, reaching levels four to five times higher and the rate of change is affected by storage temperature. In their study, these authors showed that the concentration of lactic acid was strictly dependent on time and temperature, and it was not influenced by environmental humidity or weight of the egg.

At low temperatures, the level of lactic acid remains far below the legal limit, as observed by (Hidalgo et al., 2004) for table eggs stored at 4 °C for six months, by Rossi et al. (2010), for grade B eggs stored at 4 °C for six months and by Carazzolo et al. (2002) for eggs stored at 4 °C for up to 37 days.

At higher temperatures, the results are less clear. In the study by Heaney and Curtis (1976), the level of lactic acid was still very low in eggs incubated at 37 °C for 18 days. Cattaneo and Balzaretti (1989) also observed that lactic acid remains at similar concentrations to the fresh egg after storage for six days at 37 °C. However, Stijve and Diserens (1987) observed higher concentrations in stale eggs than

in fresh ones (around 2 130 vs. 164 mg/kg of dry matter in stale and fresh eggs, respectively). Eggs kept at 20 °C for up to 37 days from lay were also shown to have a lactic acid content that increased proportionally with time (Carazzolo et al., 2002). However, even if a clear increase was shown at 20 °C, the lactic acid concentration was never higher than 500 mg/kg, which is in any case much lower than required by the legislation (1 000 mg/kg). Carazzolo et al. (2002) concluded that the level of lactic acid can be considered as a good indicator of eggs freshness, even if the legislative limit of 1 000 mg/kg of dry matter may be considered too high.

To conclude on this indicator, it appears that its concentration in shell eggs that have passed their shelf life would in any case be lower than that found in some other foods, such as fermented milk products (e.g. yogurt, containing 9 g/kg or traditional cheese with 8 g/kg) (EFSA BIOHAZ Panel and EFSA CEF Panel, 2011).

It should be noted that the assay for succinic acid, which was also required in the previous Council Directive (EC) No 89/437, is no longer in Regulation (EC) No 853/2004, probably because of variability in the results and consequent unreliability as a freshness indicator for egg products.

Uracil has been described as representing a better marker of bacterial development in pasteurized whole egg, egg yolk and egg white, based on the catalytic activity of bacterial nucleoside phosphorylases on egg's uridine (Alamprese et al., 2004; Hidalgo et al., 2004; Hidalgo et al., 2008) (see Section 12.2.3).

It appears important to notice that the egg product manufacturers may eventually test the concentration of 3-hydroxybutyric acid to assess the absence of embryonated eggs, but that they do not use the lactic acid indicator for the assessment of the microbial quality of their products.

12.2.3. Alternative methods

Alternative approaches have been developed, based on optical (red light-emitting diode light) or calorimetric measures for studying the growth of bacteria involved in the spoilage of egg products (Riva et al., 2001; Correa et al., 2008) (Table 5). Nevertheless, these methods are not relevant for routine prediction of egg product shelf lives, owing to their lack of sensitivity and the need for the manufacturer to invest in specific technical equipment.

Regarding the method targeting metabolic markers presented in Table 5, the detection of the volatile components appearing during egg product spoilage has been considered. In the USA, the acceptability of liquid egg product for consumption is based partially on odour perception by trained and licensed United States Department of Agriculture (USDA) egg product inspectors. The measurement of dimethylsulphide has been reported as an objective method for examining the acceptability of liquid or frozen egg products for human consumption, in addition to odour analysis (Brown et al., 1986). Morris et al. (1989) have shown that the chemical and organoleptic quality of pasteurized whole egg, egg white and egg yolk products starts to decrease from the fifth and the eighth day, depending on the storage temperatures of the egg products, in the range of 8-22 °C. The higher the storage temperature, the earlier the detection of atypical odours and the greater the increase in uracil concentration. It therefore appears that uracil would be a relevant marker of quality of industrial raw egg products. Therefore, uracil has been proposed as an efficient marker of bacterial development in pasteurized whole egg, egg yolk and egg white, based on the catalytic activity of bacterial nucleoside phosphorylases on egg's uridine (Alamprese et al., 2004; Hidalgo et al., 2004; Hidalgo et al., 2008).

In order to test the convenience of this putative marker of egg spoilage, odour changes were evaluated by USDA inspectors in parallel with biochemical assays of uracil concentration. The threshold for odour detection reached a concentration of around 1.7 µg uracil/g of product, convenient for the establishment of an efficient index of quality for the raw material but not suitable for the prediction of the shelf life of pasteurised egg products. The detection threshold corresponds to a heavily spoiled egg product, exhibiting a microbial load above 6 log CFU/mL, hence no longer suitable for human consumption. The convenience of other organic acids, such as acetic and succinic acids, as spoilage

markers has also been assessed. However, acetic acid was shown to exhibit similar metabolic kinetics to uracil. The detection of succinic acid, tested as a potential chemical index of the hygienic–sanitary quality of fresh pasta, was also shown to be associated to high microbial loads (9 log CFU/mL) ascribed to illegal practices of processing, i.e. the use of broken eggs and/or centrifugation (Alamprese et al., 2004).

Nowadays, the use of metabolic markers is not relevant for the evaluation of the microbial quality and freshness of pasteurized liquid egg products, within a context of improving food safety, and especially considering the products entering the composition of sensitive foodstuffs. The development of sensitive and convenient methods for early prediction of spoilage events still remains a real challenge for the egg product industry.

Concluding remarks

- The microbial quality of egg products depends on several factors, including egg freshness and quality, hygiene practices and use of appropriate processing and preservation technologies. Several methods have been developed in order to control egg freshness and quality.
- Concerning the regulation criteria, there are very few data on the quality indicators for the specific freshness criteria for egg products, as laid out in the hygiene regulations, which prevents a quantitative assessment of the evolution of the concentration of these indicators with storage time.
- In those countries where egg products are not derived from retail table eggs that have reached the sell-by date, a prolongation of the storage time of these eggs will not influence the levels of these indicators in egg products. Similarly, the impact would be minimal in those situations where eggs beyond the best-before date are used to produce egg products.
- Of the two currently recommended indicators, 3-hydroxybutyric acid is exclusively related to detection of the use of embryonated eggs, and so it is related more to fraudulent practices than to microbial growth or the conditions of storage, as its concentration is not influenced by storage time if they are not embryonated. Even if present at trace levels in infertile eggs, its concentration does not increase during storage, regardless of the storage conditions.
- Lactic acid is intended to be an indicator of microbial degradation of table eggs but it is also produced during the development of the embryo in fertile eggs. In infertile eggs, its level will be affected by the conditions of storage, mainly time and temperature.
- Lactic acid at the levels found in eggs that are past their shelf life are less than those found in some other foods, such as fermented milk products (e.g. yogurt or cheese).
- The concentration of lactic acid is not considered by the egg industry to be a useful indicator of the microbiological quality of the eggs. Microbiological and/or alternative tests are therefore used by the industry, allowing for the evaluation the microbiological quality of egg products. These analyses may allow for a better orientation of their products towards specific applications, depending on the level of the microbiological quality needed.
- Microbiological criteria are set in European legislation for egg products, i.e. a food safety criterion for *Salmonella* and a process hygiene criterion for Enterobacteriaceae at the end of the process of egg product manufacturing.

Recommendations

- Depending on the final use of the egg products, more relevant indicators of microbiological quality should be considered, for the early prediction of spoilage events and to help reduce food waste during egg product manufacture.

13. Quantitative microbial risk assessment for *S. Enteritidis* in eggs

13.1. Description of the available models

Various quantitative models have been developed in the last two decades with the scope of describing and quantifying the probability that an egg-containing serving of food is contaminated by *Salmonella* Enteritidis, its level of contamination and the consequent potential risk for humans of becoming infected via different consumption pathways. However, some of the models focus only on the prevalence of *S. Enteritidis* contamination and few of them address the issue of the pattern of microbial growth in the various production, distribution and consumption phases.

The report, '*Salmonella* in egg production in Finland: a Quantitative Risk Assessment' (Lievonon et al., 2006) examined the risk to consumers of *Salmonella* infection from table eggs in Finland. This risk assessment is based on Finnish *Salmonella* Control Programme data, surveys of consumers and institutional kitchens. The model is limited to internally contaminated eggs. It proposes a simplified risk assessment model to highlight how *Salmonella* infection in hens might be transmitted through the egg production chain up to the point of consumption. It covers consumer behaviour in relation to purchase, storage, handling, preparation and consumption. It describes the probability and number of contaminated eggs in each consumption category (private households, catering and the food industry), which are eventually converted into the number of undercooked servings potentially contaminated at the time of consumption. The risk assessment model shows that *Salmonella* prevalence in production flocks and in table eggs is exceptionally low in Finland (below 1 % in flocks). This leads to a very low level of foodborne disease caused by table eggs. As the model does not evaluate the concentration of the organism in the various stages, it is not suitable for the purposes of this opinion.

Following a qualitative evaluation, a '*probabilistic model for contamination of egg dishes with Salmonella spp. made from table eggs produced on the island of Ireland*' (Kelly et al., 2009) was developed in Ireland. This quantitative risk assessment uses some simple equations to establish the probability that an individual egg and an egg-containing food serving are contaminated with *Salmonella*. The estimate of *Salmonella* prevalence at the time of lay is based on a survey that analysed 5 018 samples of six pooled eggs for *Salmonella*. Although three main stages are covered (production and packing, distribution and storage, preparation and consumption) and various pathways are identified, they are not modelled individually to derive the probability of contamination in an individual serving. Instead an 'average' situation is considered due to the lack of reliable data. The estimates of the probability of pooling eggs and the average number of pooled eggs in the home and in the catering establishments are combined to get the probability of contamination in an egg-meal serving on the basis of the probability of contamination for a single egg. A sensitivity analysis was performed to assess the effect of the prevalence at lay and pooling on the exposure. For this purpose, a range of combined values for these two parameters were input to the model and the changes in the probability of contamination of a serving were explored. The study concluded that the public health risk is low but largely dependent on the prevalence at lay and pooling at the preparation stage. The model is not applicable to the current opinion, as only *S. Enteritidis* prevalence is addressed and not its growth in relationship to factors such as time and temperature of storage of table eggs.

The growth of *S. Enteritidis* within eggs has been modelled by the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), Health Canada, the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) and the Australian Egg Corporation Limited (AECL). All of these models are based on the assumption that no significant growth is possible until the time needed to observe a complete deterioration of the yolk membrane has passed. As explained in Section 11.2, this time is referred to as the Yolk Membrane Breakdown Time (YMT). The expected growth taking place once the membrane is degraded is then estimated. For both steps, the storage temperature and time that eggs might experience at various stages is needed as an input. Stages are, for example, production, transport, distribution, handling and storage, preparation and consumption. Eventually, different preparation and consumption pathways are considered and modelled, as they can amplify or limit the microbial growth (e.g. pooling of eggs in

the meal preparation phase can be incorporated or not). The concentration of *S. Enteritidis* is further transformed to quantify the risk for humans of getting infected by this hazard using dose–response models.

Several models have been developed by the **USDA-FSIS**, most recently in 2005 (**USDA-FSIS, 2005**). This latter model is based on an earlier model (USDA-FSIS, 1998) and covers the whole farm to fork continuum for table eggs and egg products. The model estimated the number of illnesses arising from the consumption of pasteurised and non-pasteurised table eggs (caused by *S. Enteritidis*) and pasteurised egg products (*Salmonella* spp. in this case). The 1998 model investigated production, storage and distribution, preparation and consumption as stages that can influence both the yolk membrane breakdown and the growth of *S. Enteritidis*. The microbial growth dynamics for *S. Enteritidis* were expressed as a function of the internal temperature in the eggs. Sixteen different pathways (combinations of location, pooling, use, type of cooking) were considered at the level of egg preparation and consumption. Pooling of eggs was incorporated in terms of probability of pooling and number of servings per pool separately for domestic and institutional use. It explicitly modelled the potential impact of pooling on the growth of *S. Enteritidis* (because of the post-pooling storage). The 2005 model was updated to incorporate more up to date information regarding the level of and lethality kinetics of *Salmonella* spp. in egg products and the behaviour of *S. Enteritidis* in egg yolk. In addition, the revised model incorporated the dose-response model developed by the FAO-WHO (see below for details).

In the original 1998 model, the time and temperature on farm, at retail and in the home were the most influential for the prediction of illness. The updated risk assessment focused more on the effectiveness of egg pasteurisation, which is not relevant to this assessment. Other results of interest were that refrigeration after lay at 7.2 °C would decrease significantly the number of human illnesses although the stages represented in the USDA-FSIS model do not correspond to those assessed in this Opinion.

The **FAO/WHO model (2002)** is mainly based on earlier models developed by USDA and Health Canada. This risk characterisation estimates the probability of human illness due to *S. Enteritidis* following the ingestion of a single food serving of internally contaminated table eggs, either consumed as whole eggs, egg meals or as ingredients. This farm-to-table quantitative risk assessment model covers a large number of consumption pathways. The exposure assessment model considers three stages: production, processing and distribution and preparation and consumption. It combines and modifies the USDA model mentioned above and an unpublished Health Canada risk assessment model developed in 2001 for *S. Enteritidis*, from which the structure and the shape/values given to distributions and parameters were derived. Generally, where input types were similar, the Health Canada model parameters were used. A comparative assessment of the existing dose-response models and their fit to the available epidemiological data led FAO-WHO to the choice of a single beta-Poisson dose-response function for all members of the population since it was concluded that there is insufficient evidence in the current outbreak database to conclude that some segments of the population have a higher probability of illness compared with others. Egg contamination frequencies for high- and low-prevalence flocks were modelled. Scenarios considering reduction in prevalence of flocks are included.

The impact of changing egg storage times and temperatures was simulated. It was concluded that a change of 10 % (either increase or decrease) in storage times and temperatures results in greater than a 10 % change in the predicted risk per serving.

The **Australian Egg Corporation Limited (AECL)** published ‘An egg: *Salmonella* quantitative risk assessment model for the Australian egg industry’ (Thomas et al., 2006). The model predicts how changes in industry practices may have an impact on the occurrence of salmonellosis in humans. Data on *S. Enteritidis* were used in this model to fill data gaps, as data on the serovar of interest for this assessment (i.e. *S. Typhimurium*) were scarce. A discussion of the YMT concept used by FAO and USDA is included. An alternative form of the Beta-Poisson dose-response model (named D2TAP) was used in order to better characterise the probability distributions for α and β in the model. The

exposure assessment covers from point of lay to consumption for shell and processed eggs, using the cardinal temperature equation proposed by (Rosso et al., 1993) to estimate growth rates of *Salmonella* in egg contents after the completion of the YMT. A risk characterization was established according to processing and on-farm best-, median- and worst-case scenarios. The main differences with the previous risk assessments of the USDA-FSIS and FAO-WHO are that (i) other *Salmonella* serovars associated with eggs in Australia are considered (particularly *S. Typhimurim*); (ii) data from surveys of egg production, transport and grading/processing practices are used, including temperature and time estimates gathered from point of lay to the end of wholesale storage; (iii) the influences of retail storage temperature and time on risk were explicitly considered and four storage temperatures were included in the model development (4, 16, 22 and 30 °C); and (iv) risk estimates, expressed as cases of salmonellosis per million servings, were developed for three common food types that contain eggs as a major ingredient, covering uncooked, lightly cooked and well-cooked eggs.

This risk assessment focused on the evaluation of on-farm and processing practices regarding their impact on the risk arising from the consumption of eggs. Poor practices either on-farm or during processing increase the proportion of eggs that support growth if *Salmonella* at the start of retail. Of more relevance to this Opinion is the effect of storage temperature of retail eggs on the estimated number of illnesses per million servings. The public health risk would be minimised by retail storage of eggs at 4 °C, with an eight fold increase in the risk when the eggs are stored at higher temperatures.

The Health Canada model (DeWinter et al., 2011) shares many similarities with the USDA-FSIS one. It considers different egg prevalence values based on data reported in the literature and previous assessments and a dose–response model based on a Weibull distribution. For exposure assessment it uses a simulation model originally developed in 2001 for Health Canada. The prevalence of *S. Enteritidis*-contaminated eggs among table eggs consumed is estimated from information about flock prevalence, prevalence of *S. Enteritidis* positive eggs from a positive flock, the size of positive flocks, the number of eggs produced and the fraction that are sold for the table egg market. It puts strong emphasis on the role of contaminated flocks. For storage and handling times and temperatures, it considers that time- and temperature-related conditions change the vitelline membrane's permeability and that, as mentioned earlier, *S. Enteritidis* growth does not occur until the yolk vitelline membrane breaks down and after an additional lag time. The microbial growth dynamics for *S. Enteritidis* are expressed as a function of the ambient temperature surrounding the eggs. Baseline specifications describe how production, storage and handling conditions vary among eggs. Twelve combinations of location, use and type of cooking were considered, without capturing changes in the growth though for any specific one. Temperatures through the production chain (barn, on farm, retail and consumer storage and handling) were considered at the lower and higher time and temperature settings instead of real data. It is concluded that most growth occurs during consumer storage. To address growth, conditions are represented by three categories of contaminated eggs: those that experience no growth (approximately 95.6 % at baseline conditions); contaminated eggs that experience some growth (approximately 3.8 % at baseline conditions); and those that experience growth to maximum *S. Enteritidis* density (approximately 0.6 % at baseline conditions). Egg cooking practices and egg pooling are simplified to represent the number of individuals that would consume a serving from an egg meal or recipe made with pooled eggs. Pooling of eggs is considered as the probability of pooling and number of servings per pool distinctly for domestic and institutional use, similarly to the USDA model, but it only considers the increase in the exposure due to the multiple servings made from the pooled eggs. These data have been mainly derived from expert opinion. A re-parameterised Weibull dose response function is assumed to describe the probability of illness from ingesting a dose of *S. Enteritidis*. There is a lack of sufficient information to properly describe most baseline specifications' uncertainty distributions; this makes it impossible to provide fully quantitative measures of uncertainty for the summaries provided for outputs of interest. This risk assessment is limited to grade A eggs. Various risk management strategies are evaluated to prioritize actions to control the risk of *S. Enteritidis* based on the developed models.

This model suggests that storage time and temperature at retail and at the consumer stage are the most influential ones especially when time and temperature conditions in previous stages lead to yolk

membrane breakdown. Most growth is predicted to occur during consumer storage of eggs as time–temperature combinations in the previous stages are estimated to be rarely sufficient to facilitate yolk membrane breakdown.

The models adopted by FAO-WHO, USDA-FSIS, Health Canada and AECL could all be used in principle to evaluate the impact of the prolongation of the storage of eggs. When analysed in detail, the BIOHAZ Panel selected the AECL model because it was the one that better suited the needs of the current risk assessment as this model specifically targets the impact of time and temperature on the growth of *Salmonella* in the stages post-lay. To ensure that the model was fit for purpose it was modified to include data relevant to the EU situation, as explained below.

13.2. Description of the models used

13.2.1. Modified Australian Eggs Storage Model

As explained above, a modified version of the AECL (Thomas et al., 2006) model was adopted to describe the food chain of eggs from lay to consumption. This model is referred to as the Modified Australian Egg Storage Model (MAESM). It was the one that best suited the needs of this opinion but required adaptations with respect to the following aspects:

- prevalence of eggs contaminated by *S. Enteritidis*;
- the initial number of *S. Enteritidis* present in the egg at the time of lay;
- the amount of growth occurring the first 24 hours after lay;
- ambient storage temperatures and times for on-farm, transportation and processing stages;
- inclusion of additional stages for transportation from retail to household and household storage;
- proportion of eggs refrigerated during retail and household storage;
- the maximum specific growth rate at optimum temperature used in the AECL model was modified.

The prevalence of contaminated eggs in the original AECL model was determined from Australian and international surveys of egg contamination by non-*S. Enteritidis* serovars. This approach was modified by combining European data on the flock infection prevalence, the within-flock prevalence and the rate of contaminated eggs laid by infected hens.

The Poisson distribution for the initial number of *S. Enteritidis* in the egg at the time of lay was truncated at one to avoid the possibility of no cells being present in a contaminated egg. The assumption that *S. Enteritidis* would grow in the first 24 hours after lay was removed after a re-evaluation of scientific evidence for naturally contaminated eggs.

Best, median and worst case scenarios were provided for practices on farm and during processing. For this opinion, these were not considered relevant for the terms of reference, therefore the steps from lay to retail (or wholesale in case of the catering scenarios) were considered as a single distribution of time and temperature. Distributions for the ambient storage temperatures and times for on-farm, transportation and processing stages were adjusted to reflect European conditions and practices.

Additional stages were added to the AECL model to incorporate the transportation of eggs from retail to the household and then storage in the household. The original AECL model did not explicitly consider these stages as constant storage temperature scenarios (4, 16, 22 and 30 °C) were evaluated from the start of retail storage up to 35 days. To better reflect egg storage practices in retail and households, the proportion of eggs stored under refrigeration was included in the model. Eggs which

were not refrigerated were stored under ambient room temperatures conditions. The end of the transportation stage represents the start of household storage.

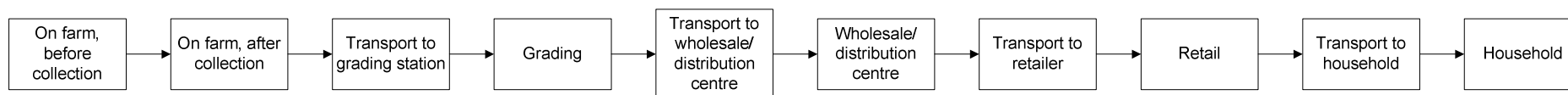
The maximum specific growth rate at the optimum temperature used in the AECL model was modified to take into consideration the following two studies published after 2006:

- a growth model for *S. Enteritidis* in egg yolk (Gumudavelli et al., 2007).
- a growth model for *Salmonella* spp. (mixed *Salmonella* strains *S. Enteritidis*, *S. Typhimurium*, *S. Blockley* and *S. Heidelberg*) in liquid whole egg (Singh et al., 2011).

The above studies showed a significant difference in the absolute growth kinetics of *S. Enteritidis* in egg yolk and liquid whole egg but a similar temperature dependence of the pathogen's growth in the two substrates. Membrane breakdown and yolk diffusion in the egg albumen is a dynamic process. Due to the limited available data it was not possible to model this process in this study. Thus, it was deemed more appropriate to describe the parameter k_{opt} in the Rosso equation using a probability distribution. The prediction ability of the cardinal model developed on the basis of the data of Singh et al. (2011) in liquid whole egg and Gumudavelli et al. (2007) in egg yolks was improved by introducing a Normal distribution with 5th and 95th percentile equal to 1.25 and 1.92, respectively, for the parameter k_{opt} (reflecting optimal growth in the two studies). A validation of the above approach against data on the growth of *Salmonella* in egg products showed a satisfactory performance. As the data in the two papers were expressed as $\ln(\text{CFU})/\text{g}$ per hour, there was a need to change the base of the log in order to be consistent with the rest of the model in which the concentration is expressed as \log_{10} (CFU). Additional details can be found in Table 10.

The MAESM model estimates the impact of storage time and temperature on the ability of *S. Enteritidis* to grow in egg contents. As indicated previously, the model excludes all stages before lay, as it is considered that possible changes in the egg shelf life will have an impact on the behaviour of *S. Enteritidis* only from this moment onwards, as all previous steps will remain unchanged. The whole process has been simplified, to allow modelling, dividing it into 10 stages as presented in Figure 7: (1) On farm, before collection; (2) On farm, after collection; (3) Transport to grading station; (4) Grading; (5) Transport to wholesale/distribution centre; (6) Wholesale/distribution centre; (7) Transport to retailer (7a: Transport to catering/food service and institutional settings); (8) Retail (8a: Catering/food service and institutional settings); (9) Transport from retail to household; (10) Household. The last two stages are relevant only for household scenarios, as for the catering distribution chain it is assumed that the eggs always come from wholesalers and not from retail establishments.

Household model



Catering/food service and institutional settings model

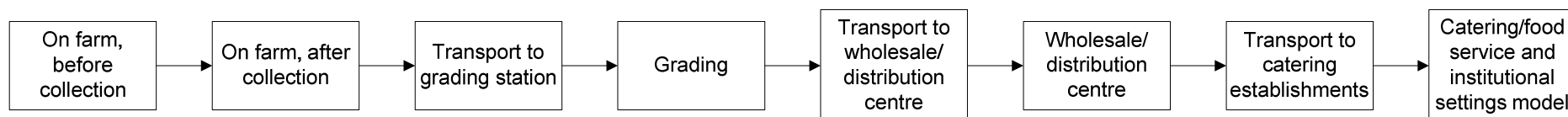


Figure 7: Schematic representation of the stages in the main model (MAESM) used in this Opinion, considering the household (top) and the catering/food service and institutional settings (bottom)

Some assumptions were made to model the process:

- Only internal contamination of eggs with *S. Enteritidis* has been considered, as it was assumed that the prolongation of storage time would lead only to a reduction of *Salmonella* counts on the eggshells and thus would not lead to an increase in the risk to humans. This is supported by the fact that *S. Enteritidis* is mainly transmitted through internally contaminated eggs and as it is the most important serovar linked to outbreaks caused by eggs or egg products (see Section 11.2 for details).
- *S. Enteritidis* will only grow in egg contents once the yolk membrane has deteriorated, and this is modelled by using the yolk membrane breakdown time, or YMT formula. This is a simplification, as *S. Enteritidis* can occasionally grow to a limited extent in egg albumen (see Sections 11.2. and 13.4.2 for additional details). The YMT was modelled using a linear relationship (Whiting et al., 2000):

$$\log_{10} \text{YMT} = a + b \times \text{temperature}$$

- This equation models the time when the eggs support growth in the albumen due to the leakage of nutrients from the yolk (see Section 11.2 for further details). The relationship between temperature and time needed to observe egg membrane breakdown is negatively correlated. For details on the parameters used for this formula, see Table 8 below.
- The expected growth of *S. Enteritidis* in the egg following the breakdown of the yolk membrane was modelled using the Rosso growth rate equation (Rosso et al., 1993; Rosso et al., 1995; Delignette-Muller and Rosso, 2000; Whiting et al., 2000). The Rosso equation (Rosso et al., 1995) belongs to the class of secondary models aiming at studying the influence of environmental factors (e.g. temperature, pH) on a set of parameters that are relevant to describe the kinetics of a microbiological process (that is the objective of a primary model). They consist of a discrete term for each environmental factor expressed as a rate (ranging between 0 and 1) towards the optimal growth for that factor. In the Rosso equation the only environmental factor is temperature, T . The expected growth rate depends on an inhibitor factor with respect to optimal conditions that is expressed as a combination of optimal, minimum and maximum growth temperature as in the following equation:

$$k = k_{opt} * \tau(T)$$

with

$$\tau(T) = \begin{cases} 0 & T < T_{min} \\ \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} & T_{min} \leq T \leq T_{max} \\ 0 & T > T_{max} \end{cases}$$

where k is the expected growth rate ((log₁₀ CFU)/g per hour) at temperature T (°C), k_{opt} is the optimum growth rate, $\tau(T)$ is a growth mitigation factor that applies when the temperature is not optimal (equal to 1 in case of $T=T_{opt}$), T_{min} and T_{max} are the predicted minimum and maximum temperatures at which growth can take place, and T_{opt} represents the temperature for optimum growth expected in the best possible conditions. It is assumed that outside the range of temperature $[T_{min}, T_{max}]$ the growth is zero.

- The distribution of the organisms in one egg is uniform. Therefore the growth rate per g and hour can be applied to the whole egg (around 50 g).

- In each stage the increase or decrease of the internal egg temperature is calculated using a cooling/warming rate per hour (see stage-dependent cooling rate constants in Table 10 and Figure 9 below) and the storage temperature using the equation:

$$T = T_s + (T_i - T_s) \exp(-c_s t_s)$$

where:

c_s is the cooling rate constant in stage s

t_s is the time spent in stage s

T_i is the internal egg temperature at the start of the time interval

T_s is the storage temperature in stage s

It was assumed that the cooling/warming rate per hour is stage-dependent but independent from the stage storage temperature.

- The number of *S. Enteritidis* at the end of each stage is obtained on the basis of the number of organisms at the start of the stage (in \log_{10} CFU), the growth rate k and the storage time t in that stage:

$$S_t = S_0 + k \cdot t$$

where:

S_t, S_0 are \log_{10} CFU of *S. Enteritidis* at the beginning and at the end of each stage

k is the growth rate expressed as ((\log_{10} CFU)/g per hour) corresponding to the storage temperature in that stage

t is the time spent in the stage

- Some parameters have been adopted from the AECL model, as it is assumed that they arise from practices that are equivalent to European ones. Examples of these are the cooling rates for eggs in the different stages, which are dependent on the type of packaging used.
- Three different cooking methods were considered in the model, in both household and catering settings: uncooked, lightly cooked and well-cooked. The risk estimates arising from these three methods are presented separately, as data were not available on the proportion of eggs that are consumed with different cooking practices at EU level. The reductions in the concentration of *S. Enteritidis* during cooking were the same as those used in the AECL model (see Table 12 for details). It is assumed that both light and thorough cooking of egg dishes is carried out in a similar fashion in the whole EU as in the United Kingdom (the original reference).
- The D2TAP dose-response model used to model the likelihood of human illness is the same as in the AECL model and is a modified version of that used in the FAO-WHO model (FAO/WHO, 2002) obtained by log transforming the alpha and beta parameters as explained in Thomas et al. (2006) because it provided a better estimate of these parameters:

$$P_{ill|cont.serving} = 1 - \left(1 + \frac{\text{Dose}}{10^{\log_{10} \beta}} \right)^{-10^{\log_{10} \alpha}}$$

where $P_{ill|cont.serving}$ is the probability of illness per *S. Enteritidis*-contaminated serving (egg).

- This model was developed using outbreak data that included different serovars and different foodstuffs. Different subgroups of the consumer population, including vulnerable groups, are considered all together, as the parameters of the dose-response model are estimated on outbreak data that includes both normal and susceptible individuals. For more information on the dose-response model, see Table 13 below.
- To increase computing efficiency, the MAESM model was run for contaminated eggs only. To obtain absolute risk estimates (i.e. for any egg, not just for contaminated ones), the probability of illness when ingesting a random serving was obtained by multiplying $P_{ill|cont.serving}$ per the prevalence of *S. Enteritidis*-contaminated eggs (details on how this was calculated are in Section 13.2.3.1). This probability was computed applying the total probability law:

$$P(A) = P(A|B) \cdot P(B) + P(A|\bar{B}) \cdot P(\bar{B})$$

where

A is the event ‘illness when ingesting a random serving’

B is the event ‘egg used in the serving is *S. Enteritidis*-contaminated’

\bar{B} is the event ‘egg used in the serving is not *S. Enteritidis*-contaminated’

As $P(A|\bar{B})$ is zero, the probability reduces to $P(A) = P(A|B) \cdot P(B)$

- Storage temperature and time were modelled using distributions based on expert opinion. The remaining distributions were adopted from the original model or based on scientific literature. The detailed list of distributions and parameters can be found in the next section (Table 10).
- Based on expert opinion, the number of eggs per serving is expected to be equal to one in EU for this assessment. The possible variability in the serving size is not reflected by a distribution.
- According to expert opinion, eggs storage time at the retail and household stages is skewed towards the early part of these stages, as it is assumed that most eggs are sold or consumed shortly after arriving at the retail establishment or the household. This is a crucial assumption in the model and was reflected in the model by using appropriate distributions as described in Table 10, and represented in Figure 8. By the prolongation of the storage time, these distributions retain their shape, but shift to the right, reflecting that the behavioural patterns of retailers and consumers are assumed to remain the same if the sell-by date or best-before date are extended.

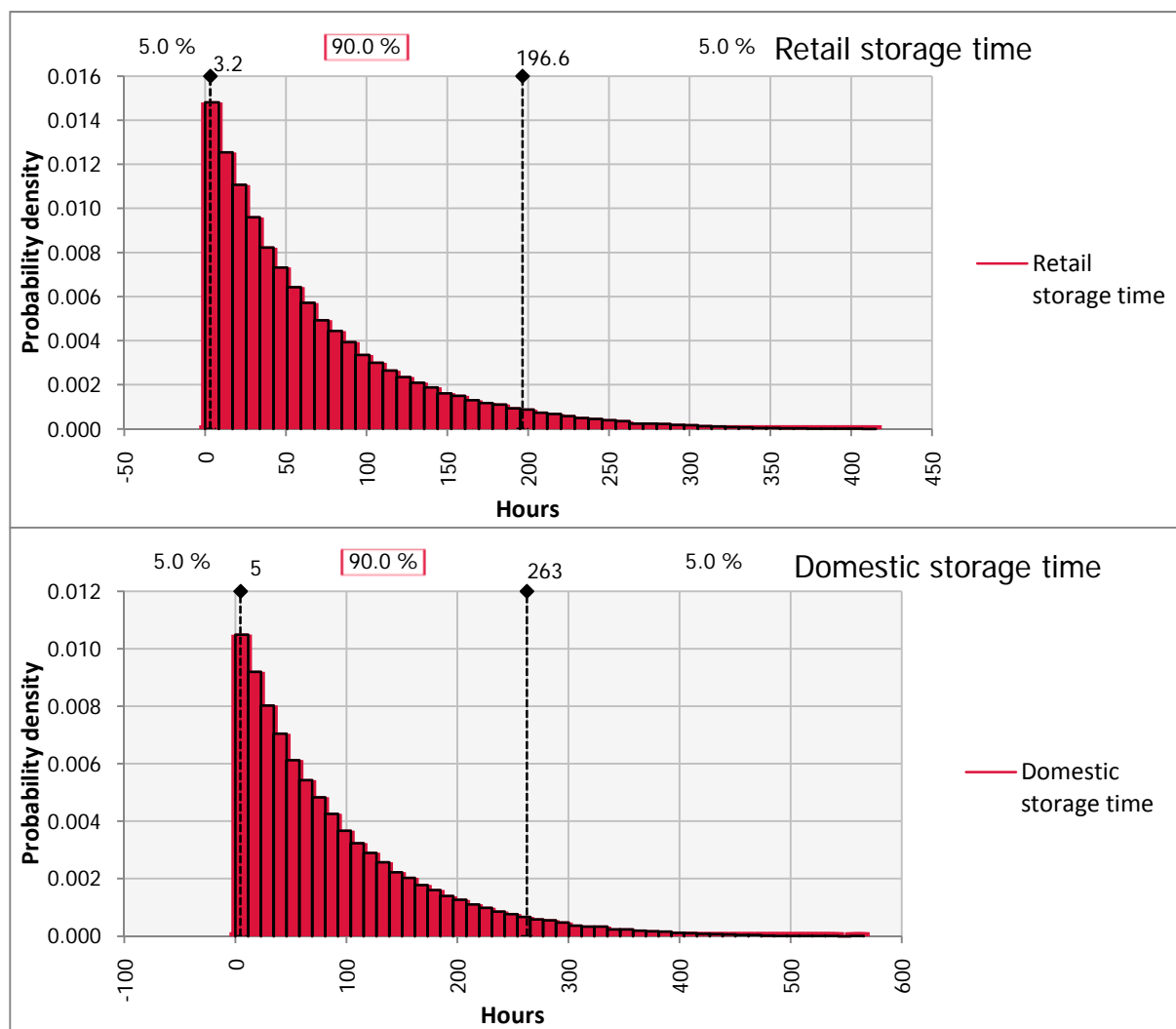


Figure 8: Distribution of the storage time at retail and in the household (in hours) to account for decreasing availability of eggs over time

The structure of the model is based on the assumption that growth is accumulated across stages, so that the total number of organisms in each egg modelled through all stages represents the cumulative effect of the conditions experienced by that egg between lay and consumption. For each of the stages in the chain represented in the model, the following process took place; the internal temperature of the egg is calculated taking into account the storage temperature and the time spent in the given stage. The adaptation of the internal temperature of the egg to that of the storage environment is computed. The internal temperature of the egg increases if the temperature of storage is greater, and decreases if it is lower. The YMT for the stage is then estimated on the basis of the internal egg temperature and the time spent in the stage. The fraction of YMT used at each stage is added to the fraction already consumed in the previous stages. When the YMT reaches a value equal or greater than one, the growth of *S. Enteritidis* is calculated for the stage (based on the temperature and time) and added to the initial concentration in egg. The process is repeated for any subsequent stages (Figure 9). The Microsoft Excel add-in software @Risk version 6.2.0 (Palisade Corporation) was used for all simulations²⁵.

²⁵ Latin Hypercube sampling of distributions with a default setting of 100 000 iterations was used for all simulations. Convergence was monitored every 500 iterations and stopped when statistics changed by <1.5 %.

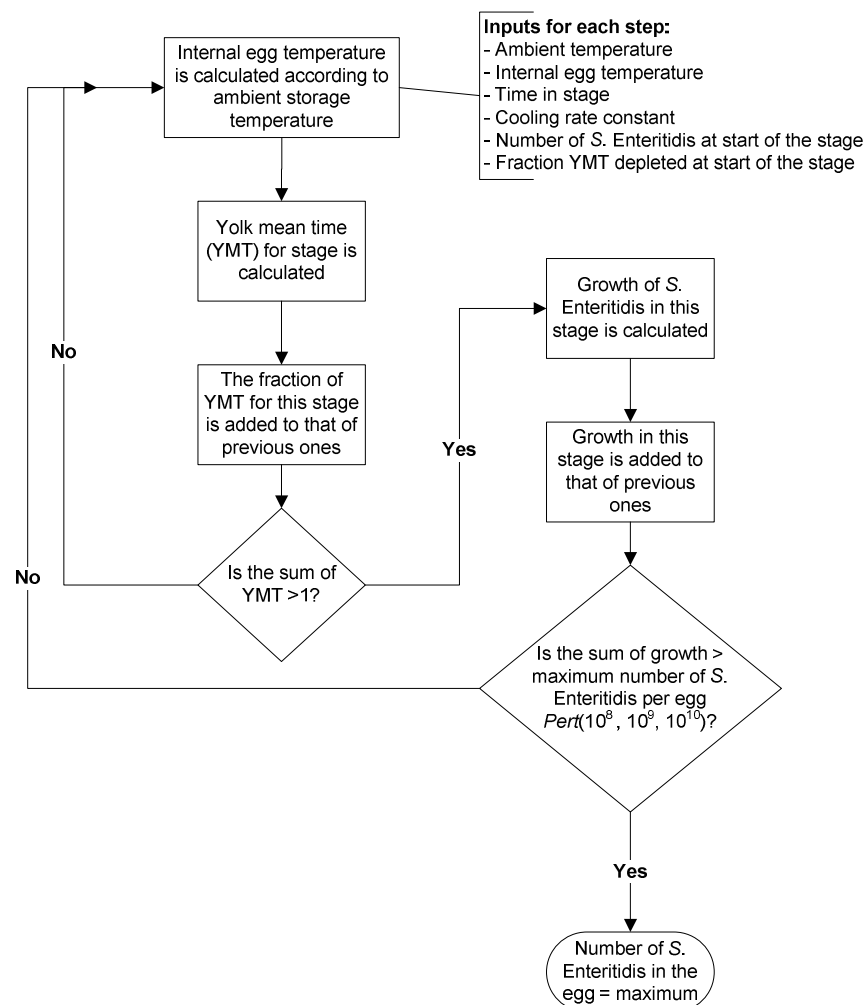


Figure 9: Process diagram for the calculation of the growth of *S. Enteritidis* in eggs for a given stage in the chain (adapted from Thomas et al. (2006), after Whiting et al. (2000))

13.2.2. Egg Pooling Module

Since the AECL model did not include the stage of egg pooling, a module (EPM: Egg Pooling Module) representing pooling practices was developed by the Assistance and Methodological support Unit of EFSA to assess the impact of the use of eggs under prolonged storage conditions for products made of pools of eggs, both in the household and in catering/other food service settings.

The EPM aims to assess whether the serving of dishes prepared with more than one egg at the household and catering/other food service settings level can change the risk of illness per serving for *S. Enteritidis* when the sell-by and best-before dates are prolonged. The module considers the steps of storage after pooling and the cooking practices to estimate the distribution of the number of *S. Enteritidis* in dishes prepared with pooled eggs and subsequently left uncooked or lightly or well-cooked. The probability of illness per serving for salmonellosis is then computed. The estimates were calculated assuming that a single contaminated egg was present in the pool. It has to be noted that the practice of pooling eggs when contaminated eggs are present leads to an increase in the prevalence of contaminated servings compared to their use as single eggs. This effect of increase of the prevalence was not investigated because it is exclusively related to the mixing of the eggs and not to the prolongation of storage (which will only influence the concentration of *S. Enteritidis* in the serving).

The initial input of the EPM is represented by the distribution of the number *S. Enteritidis* organisms expected at the end of the household/food service and institutional settings stage and estimated via the MAESM model.

The EPM replicates the structure of the main model with respect to the factors considered relevant on *S. Enteritidis* (time and temperature) and the mechanism and equation adopted for simulating its growth depending on the temperature (Rosso equation). The yolk membrane degradation intermediate step was not considered in this module since the yolk integrity is always compromised when pooling.

Both household and food service and institutional settings are modelled to assess the impact of any changes in shelf life conditions. However only the output from the worst-case scenarios from the MAESM were used, corresponding to an ambient storage temperature at retail, a sell-by date of 42 days, and best-before dates of 42, 49, 56, 63 and 70 days.

The following assumptions were made for the EPM:

- only one egg of the pool is contaminated. This is based on the assumption that eggs used for the pool are selected randomly, whereby the probability of having more than one contaminated egg in a pool is very low, based on the low prevalence of *S. Enteritidis*-contaminated eggs;
- the maximum contamination in a pool only depends on the quantity of material available to sustain the growth (i.e. number of eggs in the pool);
- the temperature of the egg pool corresponds to the storage temperature. Therefore no cooling/warming rate is considered and the interior temperature of the egg at the end of the household/catering establishment stage is not taken into consideration
- the distribution of the organisms in the pool of eggs is uniform. Therefore the growth rate per g and hour can be applied to the whole pool;
- the number of *S. Enteritidis* is evenly distributed in the pooled material. Therefore the amount of *S. Enteritidis* is equal for each serving obtained from the same pool.

13.2.3. Data and model inputs

13.2.3.1. Inputs for MAESM

The prevalence of eggs contaminated with *S. Enteritidis* was estimated by combining the flock infection prevalence, the within flock prevalence and the rate of contaminated eggs laid by infected hens. Flock prevalence estimates were obtained from harmonised monitoring data from EU MSs in 2012 (EFSA and ECDC, 2014). The within-flock prevalence and the rate of contaminated eggs laid by infected hens were based on research carried out in the United Kingdom (Arnold et al., 2014a) combined with expert opinion, as the data in the reference was considered not to fully reflect the variation in within-flock prevalence across the EU. The initial number of *S. Enteritidis* in a contaminated egg was defined using a Poisson distribution with a mean of 7 with lower bound truncated to one to account for the presence of at least one organism, to account for the variability in the numbers found in newly laid eggs and for the potential growth in the first 24 hours. The growth of *S. Enteritidis* was capped at 10^{10} CFU/egg in this model.

Data on times and temperatures of storage during the different stages were collected by means of a questionnaire submitted to competent authorities and representatives of the egg industry in several MSs. In addition, expert opinion from the Working Group and Panel was used to fill some gaps in the available data. These data might therefore not be fully representative of the actual conditions of egg storage in all MSs. The information on storage time and temperature on the stages from the farm to transport to retail is displayed in Table 6. The information for the egg distribution chain for eggs used in catering can be found in Appendix B, Table B1.

Table 6: Summary of time and temperature of storage of eggs in the EU, from the ‘on farm’ to the ‘transport to retail’ stages as derived from expert opinion (industry experts)

Stage	Time (hours)			Temperature (°C)		
	Minimum	Most likely	Maximum	Minimum	Most likely	Maximum
On farm	0	45	168	4	15	30
Transport to grading	0	6	48	4	15	30
Grading	0	18	168	5	15	30
Transport to wholesale	0	5	48	0.1	14	30
Wholesale/ distribution centre	0	23	336	0.1	13	28
Transport to retail	0	7.5	36	0	14	30

For the retail stage, the temperature was modelled as a bimodal distribution to reflect the fact that, in some MSs, refrigeration is used in retail establishments, while in other MSs this is not the case. Most likely temperature values for refrigerated and ambient storage were 4.5 °C and 19 °C, respectively. A weighting factor (expert opinion) was used to reflect the frequency of retail establishments that use each type of storage. The most likely probability of the eggs being refrigerated at retail in the EU was estimated as 15 %. As described in Section 13.2, the storage time was modelled as a decreasing exponential distribution to account for the reduction in the number of eggs with a given sell-by date available at this stage over time. More details on these distributions can be found in Table 10.

Regarding the transport from retail to the household, the temperature values were obtained by taking the average of the mean ambient temperature across EU MSs, resulting in a most likely temperature of 14 °C. For the duration of transport, the values used (10 minutes, 45 minutes and 5 hours for minimum, most likely and maximum time of transport) were obtained from a previous risk assessment on *Salmonella* in pork²⁶.

For the household stage, a similar approach to that for retail was taken. The temperature was also modelled as a bimodal distribution to reflect the fact that, although in most MSs refrigeration is used in the household for storing eggs, this is not always the case. The distribution for refrigerated storage used 5 °C and the one for storage at ambient temperature 19 °C as the most likely temperature values. The most likely probability of eggs being refrigerated was estimated as 76 %. The storage time was also modelled as a decreasing exponential distribution to account for the reduction in the number of eggs with a given best-before date that are consumed over time. More details on these distributions can be found in Table 10.

13.2.3.2. Inputs for EPM

Most of the data inputs used in the EPM are the same as for the MAESM. The inputs for the pool size in the households and in catering establishments and the post-pooling storage temperature and time were obtained from previous modelling reports (DeWinter et al., 2011). For additional details see Table 14 below.

²⁶ *Salmonella* in pork modelling report (available at www.efsa.europa.eu/en/supporting/doc/46e.pdf), see Table 10.13 on page 291 and Appendix 10.1, page 304.

Table 7: Calculation of the prevalence of *Salmonella* Enteritidis (SE)-positive eggs (contents) and growth behaviour

Parameter	Distribution/Value	Unit	Reference	Comments
Prevalence of SE positive laying flocks in the EU	0.0095	Proportion of positive flocks	EFSA and ECDC (2014)	Based on a reported prevalence of 0.95 %
Prevalence of SE infected hens in an infected flock	~Weibull(1.3812, 0.13836) with a shift of 0.0012677	Proportion of infected hens	Based on Arnold et al. (2014a) and expert opinion	Experts in the working group considered that the data in the article did not fully reflect the variation in within-flock prevalence that can be found in laying flocks across the EU. The data in the paper and the experts' opinion were therefore combined in a Weibull distribution reflecting the experts' belief. The data used to provide the estimates of the rate of <i>S. Enteritidis</i> in egg contents in the paper were combined by fitting a gamma distribution.
Rate of SE contaminated eggs laid by infected hens	~Gamma(9.523319, 0.00035828)	Proportion of contaminated eggs	Arnold et al. (2014a)	
Initial number of SE per egg, 24 h post lay	~Poisson(7) with minimum truncated to 1	CFU/egg	Thomas et al. (2006)	
Theoretical maximum number of SE per egg	~Pert(8,9,10)	log ₁₀ CFU/egg	Thomas et al. (2006)	

Table 8: Yolk Mean Time (YMT)

Parameter	Distribution/Value	Unit	Reference	Comments
YMT equation intercept	2.0872	hours	Thomas et al. (2006)	This approach to account for the effect of the yolk membrane was first proposed by Whiting et al. (2000).
YMT equation slope	−0.042579	hours	Thomas et al. (2006)	
Mean squared error	~Normal(0,0.1524)	hours	Thomas et al. (2006)	

Table 9: Growth rate equation

Parameter	Distribution/Value	Unit	Reference	Comments
Optimum growth rate, k_{opt}	$\sim Normal(1.6, 0.2)$	\log_{10} CFU/g/ hour	Singh et al. (2011); Gumudavelli et al. (2007)	Data used to estimate the normal distribution parameters were expressed as \ln CFU/g/hour. Therefore there was a need to transform the base of the logarithm from 'e' to '10'.
Minimum growth temperature	Constant(6.29)	°C	Singh et al. (2011)	
Optimum growth temperature	Constant(40.11)	°C	Singh et al. (2011)	
Maximum growth temperature	Constant(43.46)	°C	Singh et al. (2011)	

Table 10: Input data and distributions used for the egg-processing stages in the model (baseline)

Stage 1: On-farm, before collection

Parameter	Distribution/Value	Unit	Reference	Comments
Initial temperature of the egg at lay	Constant(41.2)	°C	Thomas et al. (2006)	CDF, cumulative density function
Time of day when egg is laid	Custom distribution defined on the range (6,17) with $CDF(9,11,13,15; 0.177, 0.462, 0.735, 0.93)$	hours	Thomas et al. (2006)	
Egg collection frequency	Constant(1)	times/day	Thomas et al. (2006)	
Layer shed temperature	$\sim Normal(24, 2)$	°C	Thomas et al. (2006)	
Cooling rate constant	$\sim Pert(0.8, 0.9, 1)$	hours ⁻¹	Thomas et al. (2006)	
Time to reach storeroom after collection	$\sim Uniform(1, 3)$	hours	Thomas et al. (2006)	

Stage 2: On-farm, after collection

Parameter	Distribution/Value	Unit	Reference	Comments
Storage temperature on-farm	$\sim Pert(4, 15, 30)$	°C	Expert opinion (industry questionnaires)	
Storage time on-farm	$\sim Pert(0, 45, 168)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.0528, 0.08, 0.1072)$	hours ⁻¹	Thomas et al. (2006)	

Stage 3: Transport to grading station

Parameter	Distribution/Value	Unit	Reference	Comments
Temperature during transportation	$\sim Pert(4,15,30)$	°C	Expert opinion (industry questionnaires)	
Time for transportation	$\sim Pert(0,6,48)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.0528,0.08,0.1072)$	hours ⁻¹	Thomas et al. (2006)	

Stage 4: Grading

Parameter	Distribution/value	Unit	Reference	Comments
Temperature	$\sim Pert(5,15,30)$	°C	Expert opinion (industry questionnaires)	
Storage time	$\sim Pert(0,18,168)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.0528,0.08,0.1072)$	hours ⁻¹	Thomas et al. (2006)	

Stage 5: Transport to wholesale/distribution centre

Parameter	Distribution/value	Unit	Reference	Comments
Temperature	$\sim Pert(0.1,14,30)$	°C	Expert opinion (industry questionnaires)	
Storage time	$\sim Pert(0,5,48)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.066,0.1,0.134)$	hours ⁻¹	Thomas et al. (2006)	

Stage 6: Wholesale/distribution centre

Parameter	Distribution/value	Unit	Reference	Comments
Temperature	$\sim Pert(0.1,13,28)$	°C	Expert opinion (industry questionnaires)	
Storage time	$\sim Pert(0,23,336)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.066,0.1,0.134)$	hours ⁻¹	Thomas et al. (2006)	

Stage 7: Transport to retailer

Parameter	Distribution/value	Unit	Reference	Comments
Temperature	$\sim Pert(0,14,30)$	°C	Expert opinion (industry questionnaires)	
Storage time	$\sim Pert(0,7.5,36)$	hours	Expert opinion (industry questionnaires)	Storage time
Cooling rate constant	$\sim Pert(0.066,0.1,0.134)$	hours ⁻¹	Thomas et al. (2006)	

Stage 8: Retail

Parameter	Distribution/value	Unit	Reference	Comments
Temperature (refrigeration)	$\sim Pert(0,4.5,12)$	°C	Expert opinion (industry questionnaires)	
Temperature (ambient)	$\sim Pert(14,19,25)$	°C	Expert opinion (industry questionnaires)	
Proportion refrigerated	$\sim Pert(0,0.15,0.95)$	Dimensionless	Expert opinion	
Cooling rate constant	$\sim Pert(0.066,0.1,0.134)$	hours ⁻¹	Thomas et al. (2006)	
Maximum Retail storage time	Constant(504)	hours	Set by current EU legislation	
Retail storage time	$\sim Exponential(\text{Maximum retail storage time} - \text{Age of eggs at the start of retail stage})/4$, truncated in order to assume values in the range (0, Maximum retail storage time – Age of eggs at the start of retail stage)	hours	Expert opinion	To account for the reduction of available eggs at this stage over time

Stage 8a: Catering/Food Service and Institutional settings

Parameter	Distribution/value	Unit	Reference	Comments
Temperature	$\sim Pert(0,9,28)$	°C	Expert opinion (industry questionnaires)	
Storage time	$\sim Pert(0.04,7,27)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.066,0.1,0.134)$	hours ⁻¹	Thomas et al. (2006)	

Stage 9: Transport from retail to household

Parameter	Distribution/value	Unit	Reference	Comments
Temperature	$\sim Pert(-5, 14, 32)$	°C	Expert opinion (industry questionnaires)	
Transport time	$\sim Pert(0.17, 0.75, 5)$	hours	Expert opinion (industry questionnaires)	
Cooling rate constant	$\sim Pert(0.0528, 0.08, 0.1072)$	hours ⁻¹	Thomas et al. (2006)	

Stage 10: Household

Parameter	Distribution/value	Unit	Reference	Comments
Temperature (refrigeration)	$\sim Pert(2, 5, 12)$	°C	Expert opinion (industry questionnaires), Marklinder et al. (2004); Cibir et al. (2012)	Data from Marklinder et al. (2004) and Cibir et al. (2012) were used for the maximum estimate of refrigeration temperature in the household
Temperature (ambient)	$\sim Pert(14, 19, 30)$	°C	Expert opinion (industry questionnaires)	
Proportion refrigerated	$\sim Pert(0.2, 0.76, 1)$	Dimensionless	Expert opinion	
Cooling rate constant	$\sim Pert(0.066, 0.1, 0.134)$	hours ⁻¹	Thomas et al. (2006)	
Maximum shelf life of eggs at baseline	Constant(672)	hours	Set by current EU legislation	Best-before date
Domestic storage time	$\sim Exponential(\text{Maximum domestic storage time} - \text{Age of eggs at the start of domestic stage 10})/4$, truncated in order to assume values in the range (0, Maximum domestic storage time – Age of eggs at the start of household stage)	hours	Expert opinion	To account for the reduction of available eggs at this stage over time

Egg processing stages in the MAESM model (alternative scenarios)

(a) Consumption in the household

The total storage time was extended in the model to a maximum best-before date of 70 days in weekly intervals. It is assumed that an extension of the sell-by and best-before dates would not affect practices earlier in the egg-processing chain. The current sell-by date of 21 days will be replaced by 28, 35 and 42 days, respectively. The remaining time up to 70 days will be taken up by the household stage. Figure 10 displays a schematic representation of these scenarios, and Table 11 below provides more details about the different sell-by and best-before dates that were evaluated.

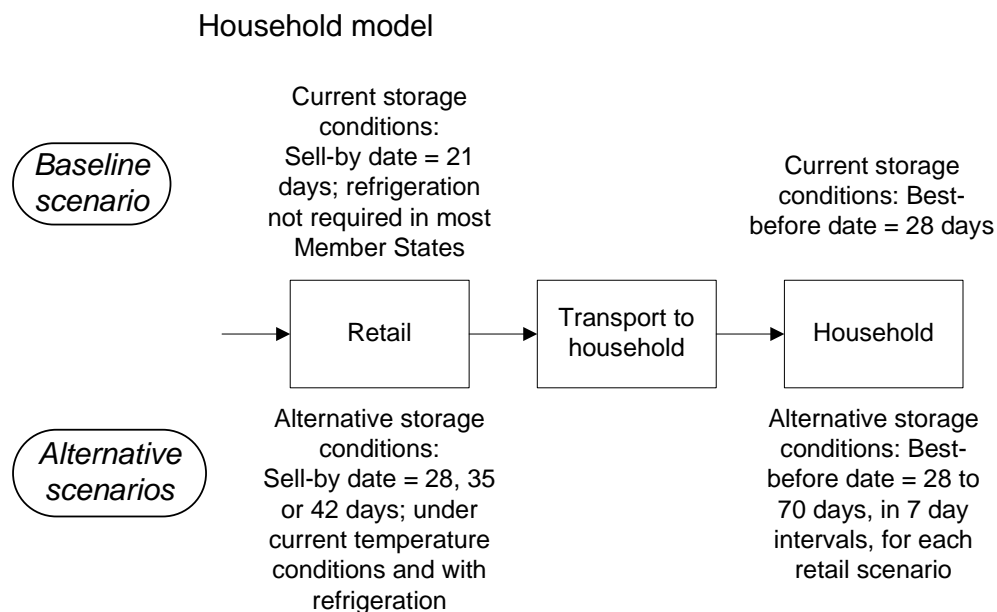


Figure 10: Baseline and alternative scenarios for the household egg distribution chain

The temperatures used at retail for these alternative scenarios will be (i) the same as in the baseline (i.e. a bimodal distribution as per stage 8 in Table 10 above); and (ii) a refrigeration-only temperature ($Pert(0,4.5,12)$).

(b) Consumption in catering/food service and institutional settings

The time of storage in catering/food service and institutional settings will be equally extended to a maximum best-before date of 70 days in weekly intervals. It is therefore assumed that the extension of the sell-by and best-before dates would not affect practices in the egg-processing chain. Figure 11 displays a schematic representation of these scenarios, and Table 11 below provides more details about the different sell-by and best-before dates that were evaluated. The temperatures used for these alternative scenarios will be the same as in the baseline.

Catering/food service and institutional settings model

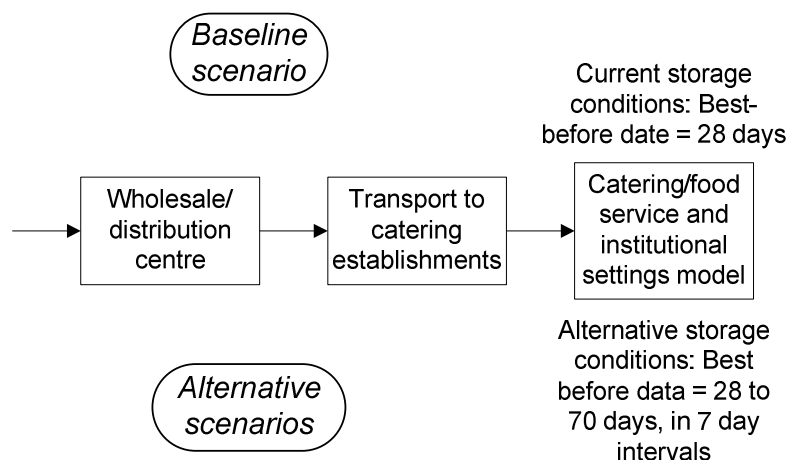


Figure 11: Baseline and alternative scenarios for the catering egg distribution chain

Table 11: Dates used in the model for the baseline and alternative scenarios. For catering, only the scenarios for best-before dates apply.

Days post lay Scenarios	Sell-by date (retail)				Best-before date (household/catering)					
	21	28	35	42	28	35	42	56	63	70
Baseline	●				●					
Alternative 1		●			●	●	●	●	●	●
Alternative 2			●			●	●	●	●	●
Alternative 3				●			●	●	●	●
Alternative 4		◇			●	●	●	●	●	●
Alternative 5			◇			●	●	●	●	●
Alternative 6				◇			●	●	●	●
Worst-case scenario				●						●

● Scenarios with egg storage at retail under current conditions

◇ Scenarios with egg storage under refrigeration conditions in all retail establishments

Table 12: Preparation and consumption inputs

Parameter	Distribution/ value	Unit	Reference	Comments
Uncooked	Constant(0)	\log_{10} CFU (reduction)	Thomas et al. (2006)	
Lightly cooked	$\sim Normal(2,0.5)$	\log_{10} CFU (reduction)	Thomas et al. (2006)	The definitions of lightly cooked are as described by Bates and Spencer (1995) and Humphrey et al. (1989b): boiled 4 minutes, fried ('sunny side up') or microwaved.
Well-cooked	$\sim Normal(12,1)$	\log_{10} CFU (reduction)	Thomas et al. (2006)	The definitions of well-cooked are as described by Bates and Spencer (1995) and Humphrey et al. (1989b): hard boiled or scrambled, cooking as for cakes and biscuits

Table 13: Dose–response inputs

Parameter	Distribution/value	Unit	Reference	Comments
$\log_{10} \beta$ parameter	$\sim Normal(1.727,0.227)$	N/A	Thomas et al. (2006)	A correlation between $\log_{10} \beta$ and $\log_{10} \alpha$ of 0.892 is used' for the dose response model
$\log_{10} \alpha$ parameter	$\sim Normal(-0.871,0.089)$	N/A	Thomas et al. (2006)	

Table 14: Inputs for the pooling module

Parameter	Distribution/value	Unit	Reference	Comments
Initial numbers of <i>S. Enteritidis</i> (SE) per egg	Output of MAESM as number of SE/egg derived from: stage 8 for catering, and stage 10 for home	CFU/egg	Thomas et al. (2006)	Input variable from main model
Pool size	$\sim Pert(25,50,75)$ rounded to integer for catering $\sim Pert(1,6,12)$ rounded to integer for household	N° eggs	DeWinter et al. (2011)	
Maximum number of SE per egg	$\sim Pert(10^8, 10^9, 10^{10})$ rounded to integer	$\log_{10} CFU \text{ egg}^{-1}$	Thomas et al. (2006)	
Maximum number of SE in the pool	(Maximum number SE egg^{-1}) \times (size of pool)	$\log_{10} CFU \text{ pool}^{-1}$	Function	See Table 7 for maximum number of SE per egg
Storage time post pooling	$\sim Pert(1,3,24)$ for catering $\sim Pert(1,3,8)$ for household	hours hours	DeWinter et al. (2011)	
Storage temperature post pooling	$\sim Pert(15,20,25)$ for baseline scenario	°C	DeWinter et al. (2011)	
Optimum growth rate, k_{opt}	$\sim Normal(5^{th} -le = 1.25; 95^{th} -le = 1.92)/ln(10)$	$\log_{10} CFU/g/ \text{ hour}$	Singh et al. (2011); Gumudavelli et al. (2007)	See main model
Minimum growth temperature, T_{min}	Constant(6.29)	°C		
Optimum growth temperature T_{opt}	Constant(40.11)	°C		
Maximum growth temperature T_{max}	Constant(43.46)	°C		
Growth rate	Same as in main model	$\log_{10} CFU \text{ hour}^{-1}$		Rosso equation (see main model)
Serving portion	Constant(50)	mL	Expert opinion	
Volume of egg	Constant(50)	mL	Expert opinion	
Serving per pool	Pool size	number of servings pool^{-1}		
Preparation (Uncooked)	Constant(0)	$\log_{10} CFU$ (reduction)	Thomas et al. (2006)	
Preparation (Lightly cooked)	$\sim Normal(2,0.5)$	$\log_{10} CFU$ (reduction)	Thomas et al. (2006)	
Preparation (Well-cooked)	$\sim Normal(12,1)$	$\log_{10} CFU$ (reduction)	Thomas et al. (2006)	
Dose–response: $\log_{10} \beta$ parameter	$\sim Normal(1.727,0.227)$	N/A	Thomas et al. (2006)	
Dose–response: $\log_{10} \alpha$ parameter	$\sim Normal(-0.871,0.089)$	N/A	Thomas et al. (2006)	

13.3. Results of the modelling

13.3.1. Prevalence of eggs internally contaminated with *S. Enteritidis*

The rate of eggs laid in the EU that are internally contaminated with *S. Enteritidis* was estimated by combining the estimated laying flock prevalence of *S. Enteritidis* in the EU (0.95 %, EFSA and ECDC, 2014), the within-flock prevalence (Table 7), and the rate of eggs internally contaminated with *S. Enteritidis* laid by infected hens (Table 7). Figure 12 shows the output of this simulation, with an estimated mean prevalence of *S. Enteritidis* internally contaminated eggs of 0.04 %.

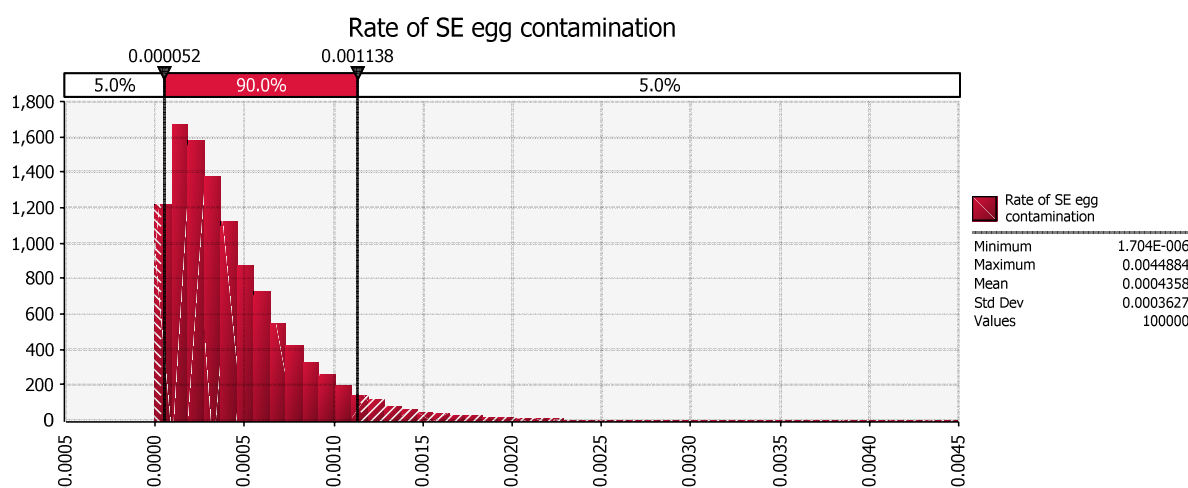


Figure 12: Estimate of the prevalence of eggs internally contaminated with *S. Enteritidis*

13.3.2. Household

As explained in Section 13.2 above, the assessment of an extension of the storage time for eggs was explored using the MAESM model by combining three potential sell-by dates (under the same temperature conditions and with refrigeration at retail) with up to seven best-before dates. The risk was expressed in absolute terms as the number of illnesses per million servings of uncooked and lightly cooked egg meals, with each serving being equivalent to a single egg. In addition, and to allow for an easier comparison with current storage conditions, the relative risk of illness was also used, calculated by dividing the absolute risk in the alternative scenario by that of the baseline scenario (i.e. corresponding to the current situation). For well-cooked egg meals, there is no change in the absolute risk of number of illnesses as this is very small in both the baseline and the alternative scenarios, therefore these results were not presented, although some scenarios including well-cooked egg meals are included in Appendix B.

The evolution of the percentage of eggs that have the yolk membrane broken along the egg distribution chain was also estimated for both the baseline scenario and the worst case scenario. The latter considers current storage temperatures in retail and households and extends the sell-by date to 42 days and the best-before date to 70 days. In addition, this worst-case scenario considering instead refrigeration during retail was assessed. The percentage of eggs in which the yolk membrane becomes dysfunctional, allowing leakage of yolk nutrients, increases along the distribution chain, starting from 0 % at lay, increasing to approximately 9 % at retail and 16 % at household level, in the current situation (baseline). In the worst-case scenario, the proportion of eggs with dysfunctional yolk membranes increases more rapidly; when the storage time is prolonged by extending the sell-by date to 42 days and the best-before date to 70, the proportion of eggs with dysfunctional yolk membrane increases to around 25 % during retail and to almost 50 % at household level. Refrigeration during retail for the same worst-case scenario would reduce the proportion of eggs with a dysfunctional yolk membrane at retail, only slightly compared to the baseline scenario, and more dramatically compared

to the worst-case scenario without refrigeration. At household level, the effect of refrigeration at retail is less pronounced, resulting in a proportion of eggs with dysfunctional yolk membrane twice that of the baseline scenario, and around 16 % less compared to the scenario without refrigeration at retail. Results are presented in Figure 13.

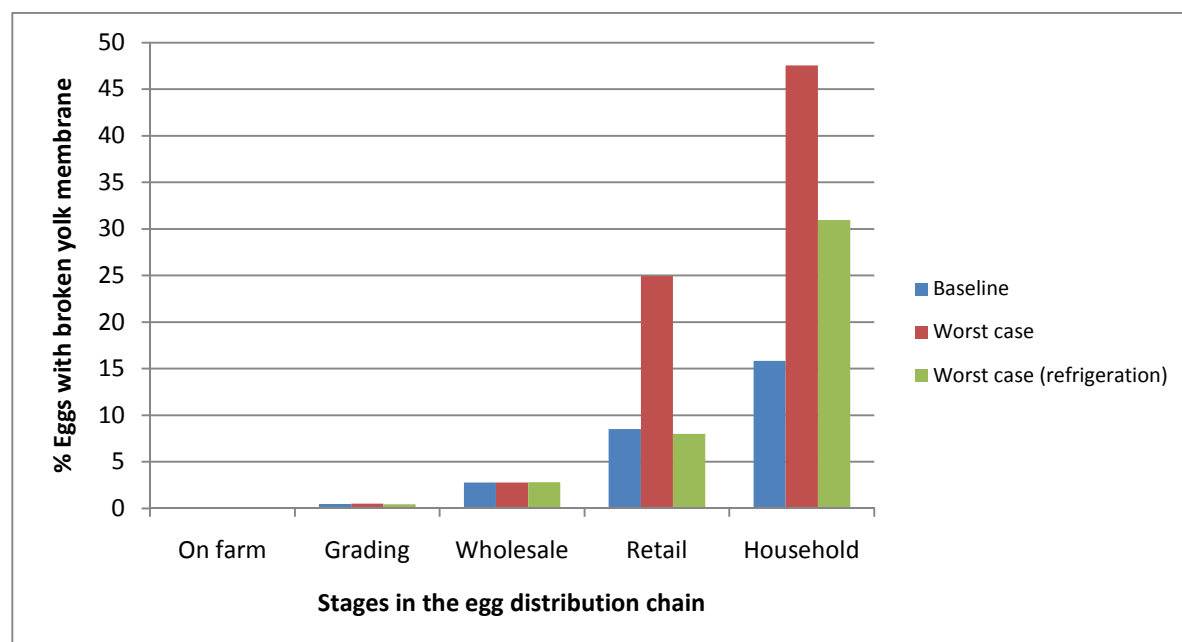


Figure 13: Evolution of the percentage of eggs in which the yolk membrane becomes dysfunctional along the egg distribution chain in eggs under current storage conditions (baseline) and the worst case scenario (sell-by date 42 days and best-before date 70 days, with and without refrigeration during retail)

Figure 14 displays the estimated mean number of illnesses per million servings for uncooked and lightly cooked egg meals under several scenarios of storage time, at current storage temperatures (both in retail and at the household). The baseline scenario estimates 43 and 30 cases of salmonellosis per million servings for uncooked and lightly cooked egg meals, respectively. Prolonging of the storage time of table eggs is expected to result in an increase in the number of illnesses per million servings for both uncooked and lightly cooked egg meals but not when eggs are well-cooked (see Appendix B for further information on well-cooked egg meals). The magnitude of this increase depends on the additional storage time that the eggs spend at both retail and household. To make these figures more meaningful, it is worth considering the amount of eggs consumed in the EU. For example, according to data from the International Egg Commission²⁷, in Portugal the egg consumption per capita per annum is 186, whilst in France this figure is 248. Extrapolating these figures for the whole of the EU (503 million inhabitants²⁸) results in between 93 558 and 124 744 million eggs consumed in the EU each year. As mentioned earlier, data on cooking and consumption of different egg meal types were not available at EU level. A recent Dutch study suggests that the proportion of consumers that eat raw eggs is very low (Chardon and Swart, 2012), so this could help to put these figures in context.

For example, extending only the sell-by date by one week (from 21 to 28 days) is estimated to result in 61 and 45 cases of salmonellosis per million servings for uncooked and lightly cooked egg meals respectively, which means an increase of 18 and 15 cases compared to the baseline, and a relative risk of illness of 1.4 and 1.5. If the best-before date is also extended by one week (from 28 to 35 days), the number of cases per million servings for uncooked and lightly cooked egg meals would be 68 and 51, with a relative risk of 1.6 and 1.7. In the worst case scenario considered in this assessment (sell-by

²⁷ <https://www.internationalegg.com/corporate/eggindustry/details.asp?id=18>, accessed 1 July 2014

²⁸ http://europa.eu/about-eu/facts-figures/living/index_en.htm, accessed 1 July 2014

date of 42 days, best before date of 70 days), such figures would be 127 and 104 (84 and 74 additional cases for uncooked and lightly cooked egg meals compared to the baseline). The relative risk of illness in this case would be: 2.9 and 3.5. The results of all alternative scenarios for absolute and relative risk estimates are reported below in Figures 14 and 15 respectively. The observed higher relative risk for lightly cooked egg meals as compared to uncooked egg meals, is considered to be a result of a less efficient log reduction because of the higher content of *S. Enteritidis* in the eggs before lightly cooking them i.e. the change in dose between lightly cooked and uncooked egg meals differs resulting in a relative higher risk for lightly cooked eggs. The absolute risk is obviously still higher for uncooked egg meals.

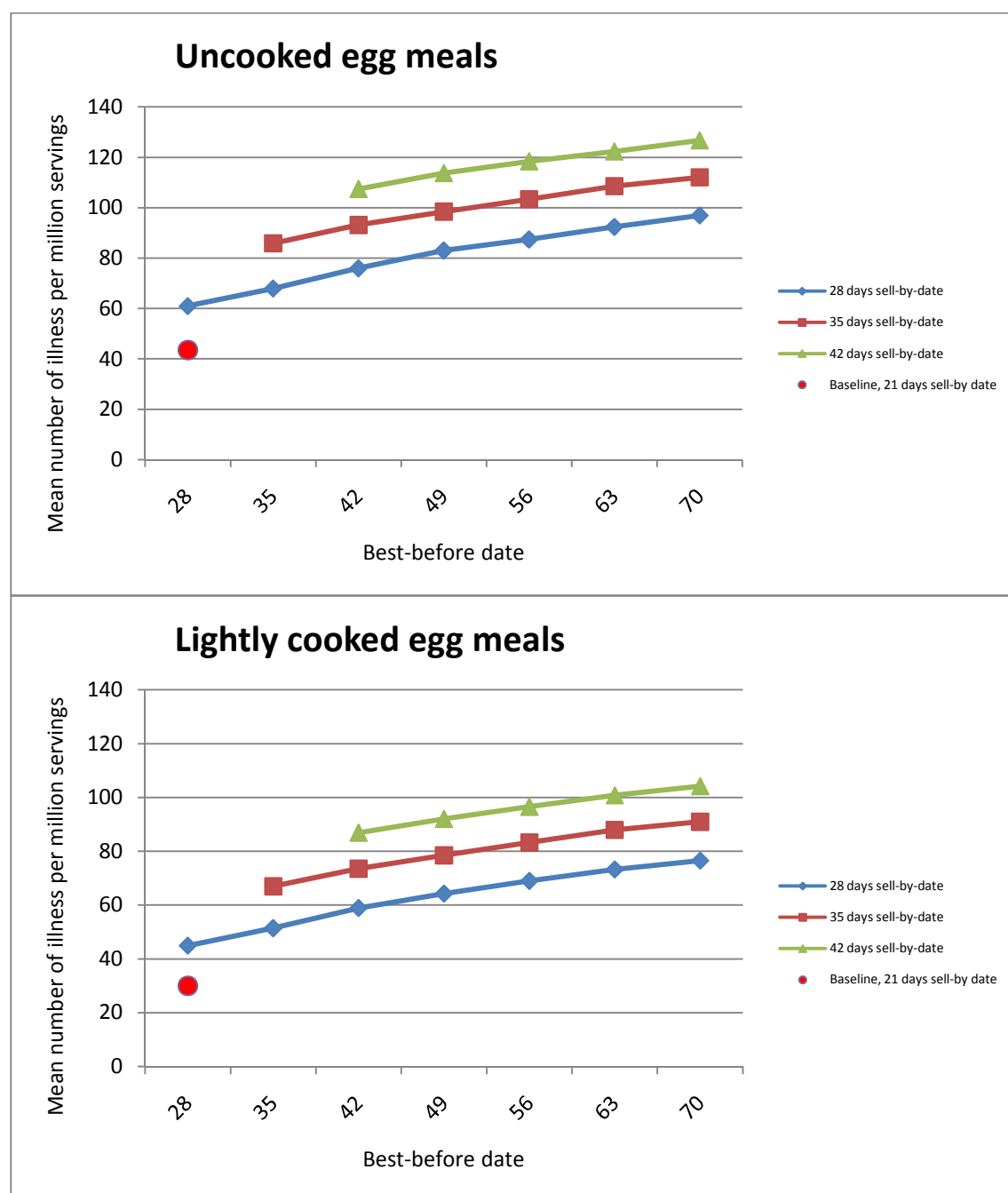


Figure 14: Mean number of illnesses per million servings of uncooked and lightly cooked egg meals under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions)

The starting points of the lines in Figure 14 represent the scenarios where the sell-by date is the same as the best-before date, that is, equivalent to the elimination of the sell-by date. Table 15 includes the estimates of the mean number of illnesses per million servings for these initial values. These figures show that the risk (or number of illnesses) increases faster when extending the storage time in retail compared to a similar extension of storage in the household. For example, for uncooked egg meals, 30 additional illnesses would occur if the sell-by date changed from 28 to 35 days, while only 15 additional cases would result from keeping the eggs an extra week in the household (i.e. keeping the sell-by date fixed at 28 days but increasing the best before date to 35 days). This difference is due to the eggs being refrigerated more often in the household (estimated at 76 %) than in retail establishments (15 %).

Table 15: Mean number of illnesses per million servings of uncooked and lightly cooked egg meals under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions)

Scenarios considered	Uncooked egg meals	Lightly cooked egg meals
Baseline (21 days sell-by date, 28 best-before date)	43.5	30.0
28 days sell-by/best-before date	61.0	45.0
35 days sell-by/best-before date	85.9	67.
42 days sell-by/best-before date	107.4	86.9

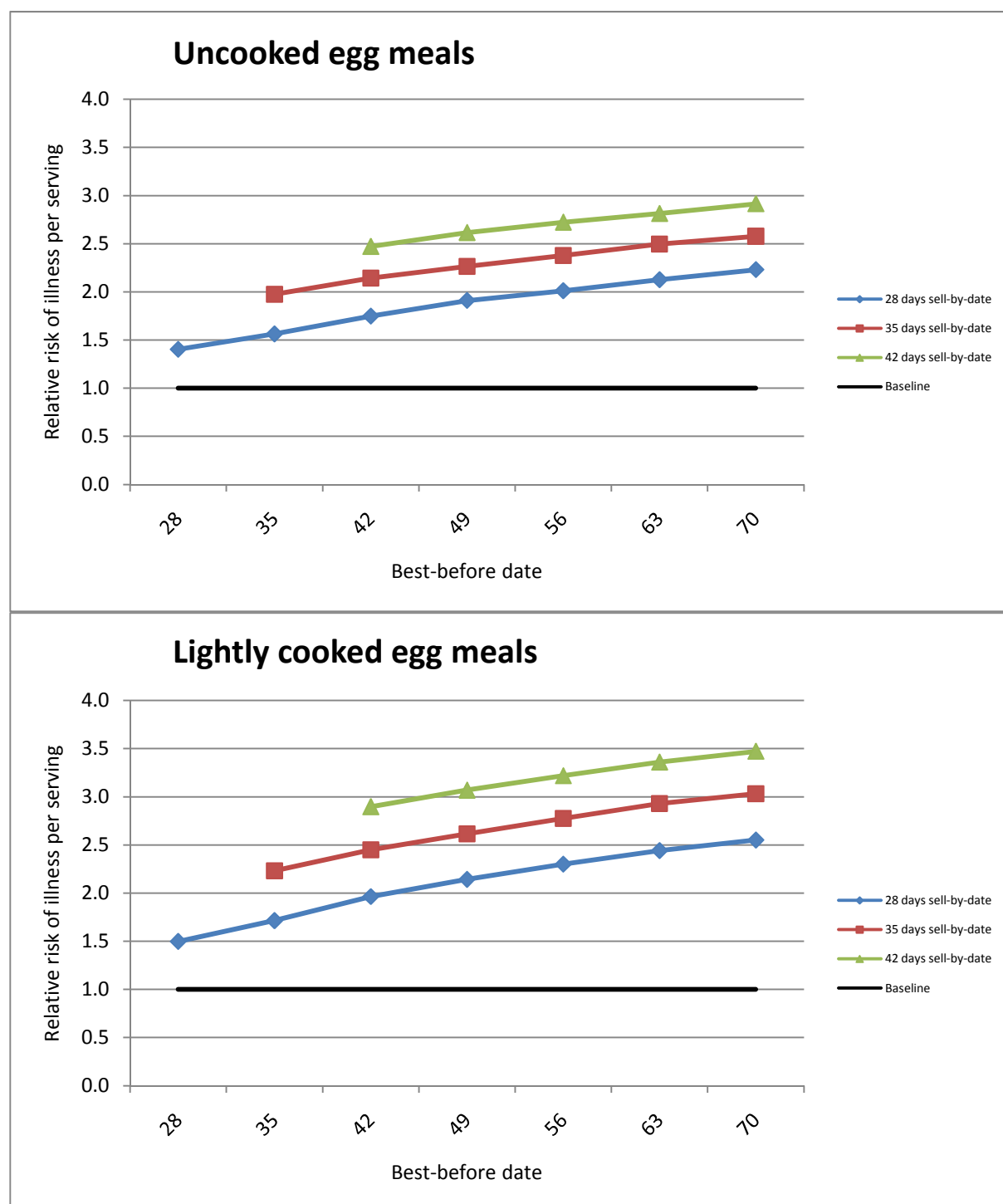


Figure 15: Relative risk of illness from uncooked and lightly cooked egg meals under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions). The risk was calculated as the mean number of illnesses per serving

The implementation of refrigeration as currently used in the EU (with temperatures ranging from 0 to 12 °C) during the retail stage limits to some extent the increase in the risk arising from the prolongation of the storage time, but in most cases the resulting risk is still greater than the estimate for the current situation. The risk is reduced in the case of a prolongation of up to three weeks in the sell-by date, and also of one week or two weeks for the best-before date for a sell-by date of 35 and 28 days respectively. If refrigeration is applied during storage, the risk is reduced when compared with

the current situation, but if the sell-by date or the best-before date are prolonged further, the risk estimate is greater, although at a lower rate than in the absence of refrigeration. Therefore, the longer the sell-by-date (beyond an extension of three weeks) or best-before date beyond two weeks, the higher the risk even under refrigeration conditions at retail (Figures 14, 15, 16 and 17). Additional information from the modelling outputs can be found in Appendix B.

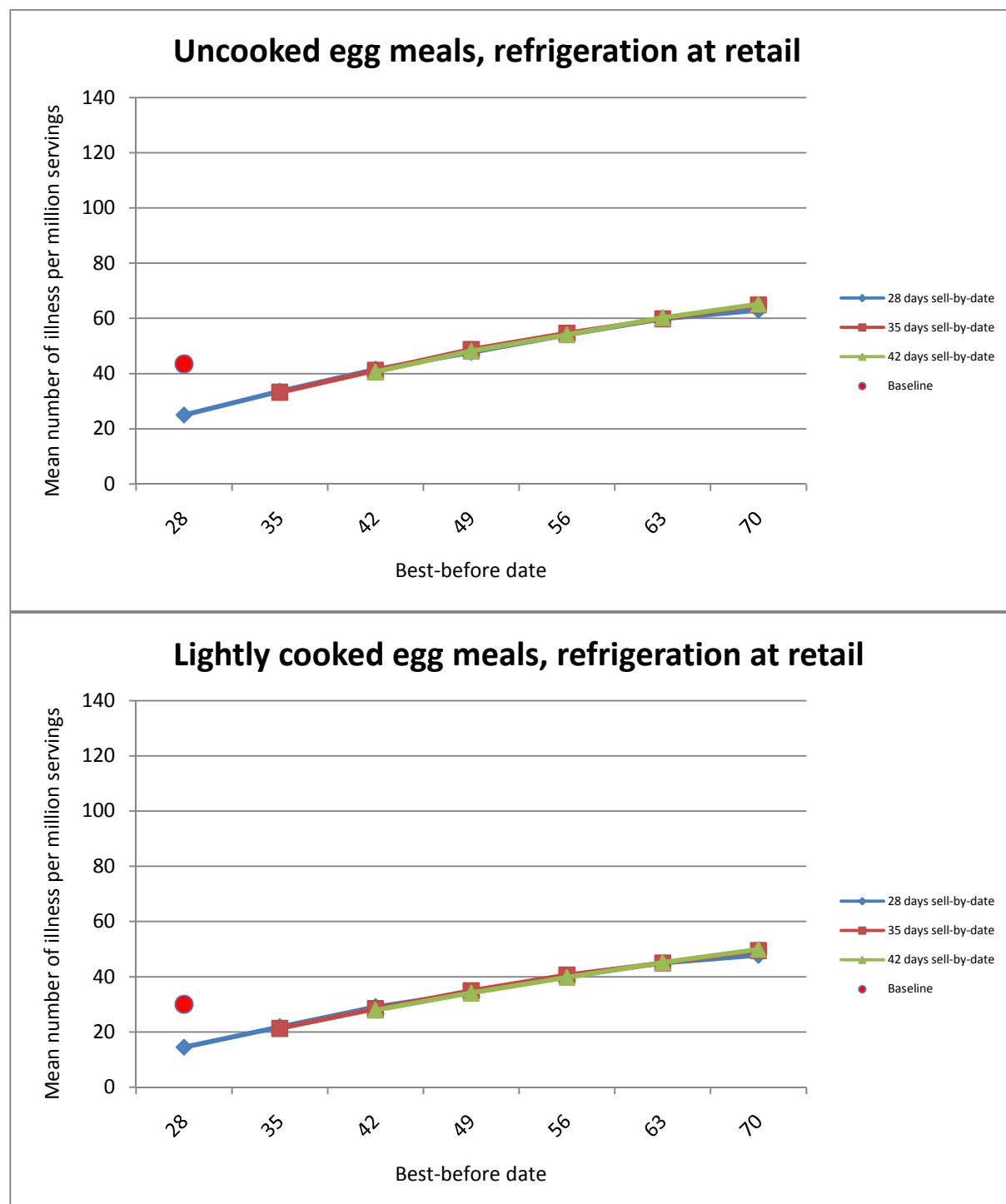


Figure 16: Mean number of illnesses per million servings of uncooked and lightly cooked egg meals under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions), with refrigeration during storage at retail

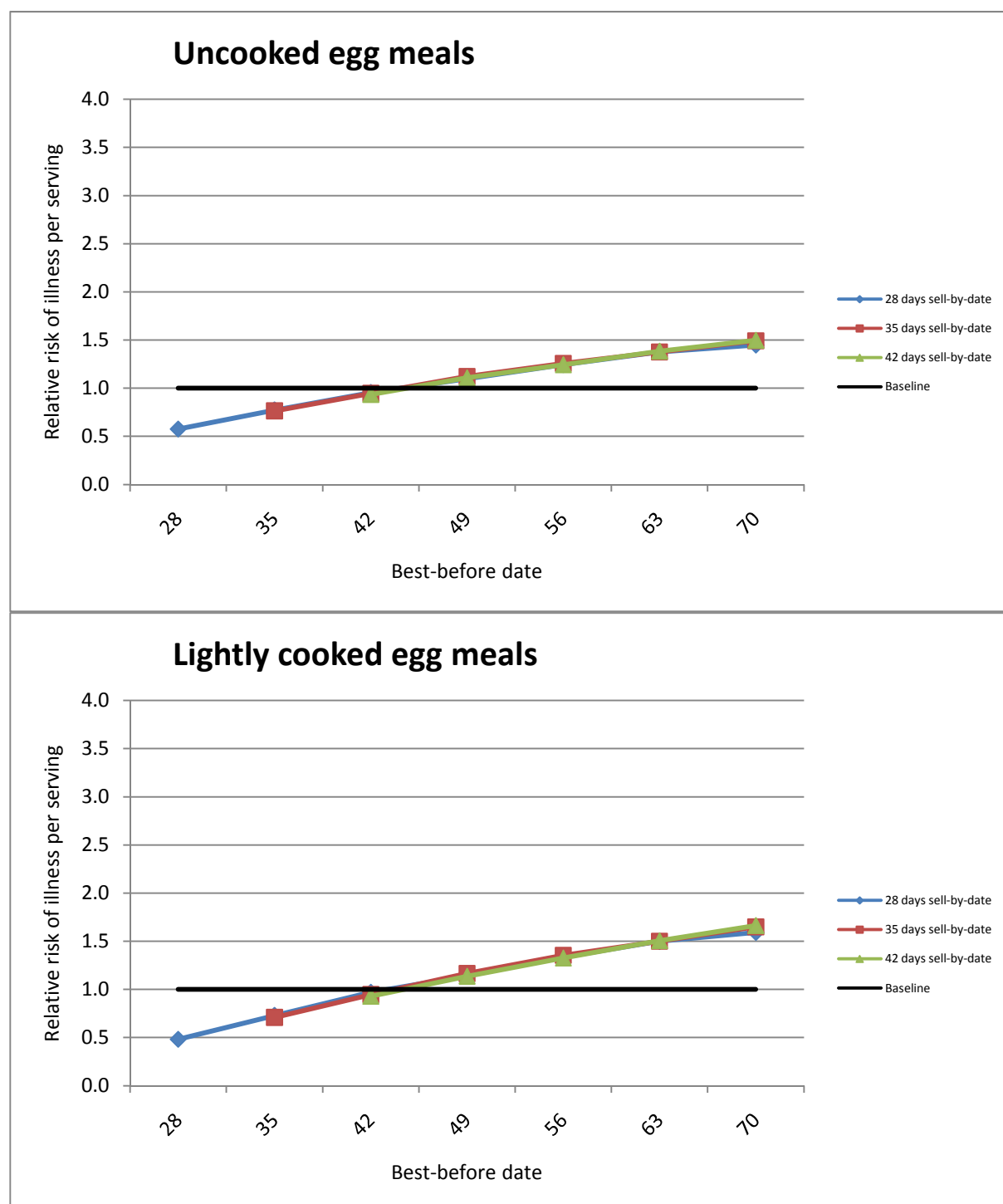


Figure 17: Relative risk of illness from uncooked and lightly cooked egg meals under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions), with refrigeration during storage at retail. The risk was calculated as the mean number of illnesses per serving

13.3.2.1. Pooling example - household

As described in section 13.2.2, the EPM was developed to explore the influence of pooling practices on the risk arising from prolonged egg storage, when only one of the eggs used in the pool was contaminated with *S. Enteritidis*. For this purpose, the baseline scenario (i.e. sell-by date of 21 days and best-before date of 28) was compared to the ‘worst-case scenario’ of extending the sell-by date to

42 days and the best-before date in weekly intervals to 70 days. The relative risk of illness estimates show an increase of the risk with storage time (Figure 18), similar to that observed in individual eggs (Figure 15).

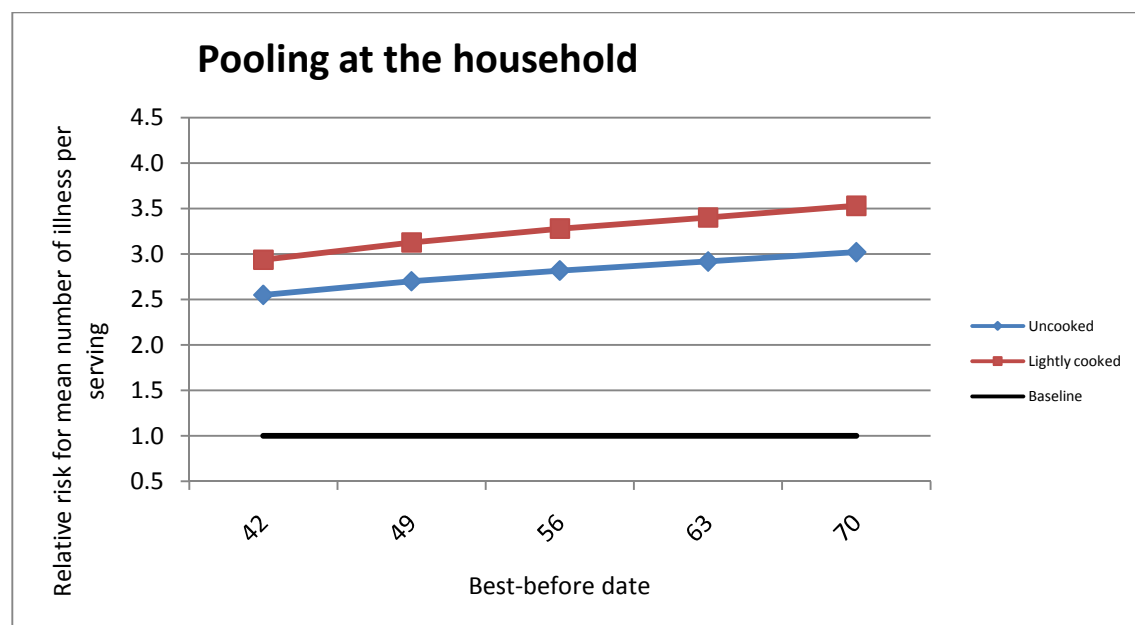


Figure 18: Relative risk of illness of uncooked and lightly cooked egg meals made with pooled eggs at the household when only one of the eggs used in the pool was contaminated with *S. Enteritidis*, under different alternative storage conditions to those from the baseline scenario (i.e. current egg storage conditions). The risk was calculated as the mean number of illnesses per serving

As explained in Section 13.2.2, pooling eggs including a contaminated egg would increase the prevalence of contaminated servings compared to their individual use. This impact was not estimated since it was not the focus of this assessment, as this would happen irrespective of the changes of storage time. Nevertheless, to account for the increased probability of illness for consumers of servings derived from the contaminated pool, the risk was also expressed as the probability of at least one of the consumers exposed to the contaminated pool servings becoming sick. For the household setting, the number of exposed individuals was set at six, the same as the most likely number of eggs used in the pool. The probability of at least one of the six people becoming ill arising from consumption of uncooked dishes rises from 0.47 under baseline conditions to between 0.83 to 0.89, depending on the prolongation of the best-before date. For lightly cooked eggs the probability is slightly lower (from 0.36 to between 0.75 and 0.82) (Figure 19).

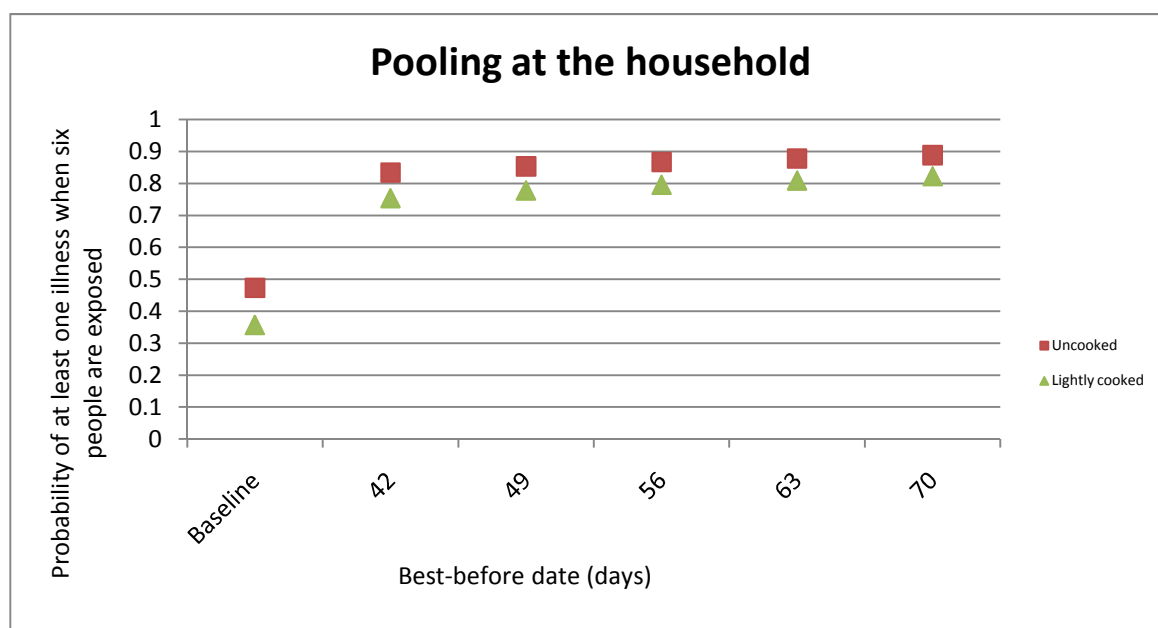


Figure 19: Probability of at least one illness when six people are exposed to uncooked or lightly cooked egg meals made with pooled eggs at the household when only one of the eggs used in the pool was contaminated with *S. Enteritidis*, under different alternative storage conditions to those from the baseline scenario (i.e. current egg storage conditions)

As with individual eggs, well-cooked egg meals made from pooled eggs did not represent a significant risk to consumers (around 1 to 4 cases per million servings from contaminated pools). The risk would therefore be negligible or extremely low, particularly if the very low prevalence of contaminated eggs is taken into account. Therefore only the results for uncooked and lightly cooked meals are presented.

13.3.3. Catering/food service and institutional settings

The modelling exercise for these establishments is somewhat simpler than in the household setting given the assumption that eggs are obtained directly from wholesalers, hence the absence of the retail stage. For this reason, the alternative scenarios explored only consider an extension of the best-before date. As for the household, results are presented in absolute terms as the number of illnesses per million servings of uncooked and lightly cooked egg meals, as well as the relative risk of illness.

Similarly to results obtained in the household setting, a prolongation of storage time of eggs used in catering/food service and institutional settings would lead to an increase in the risk of salmonellosis. The calculated mean number of illnesses would increase from 24 (uncooked egg meals) and 13 (lightly cooked egg meals) cases of illness per million serving in the baseline scenario, to 32 and 19 cases of salmonellosis with a prolongation of one week of the best-before date, respectively. The results of all alternative scenarios for absolute and relative risk estimates are reported below in Figures 20 and 21 respectively.

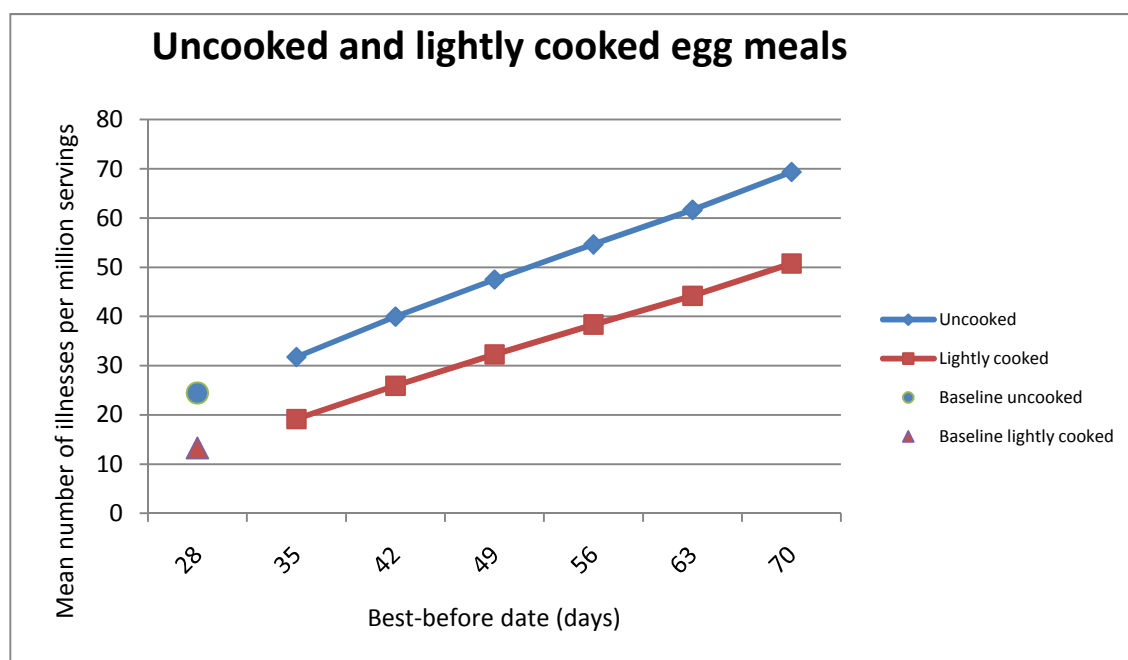


Figure 20: Mean number of illnesses per million servings of uncooked and lightly cooked egg meals under alternative storage conditions in catering establishments different from those of the baseline scenario (i.e. current egg storage conditions)

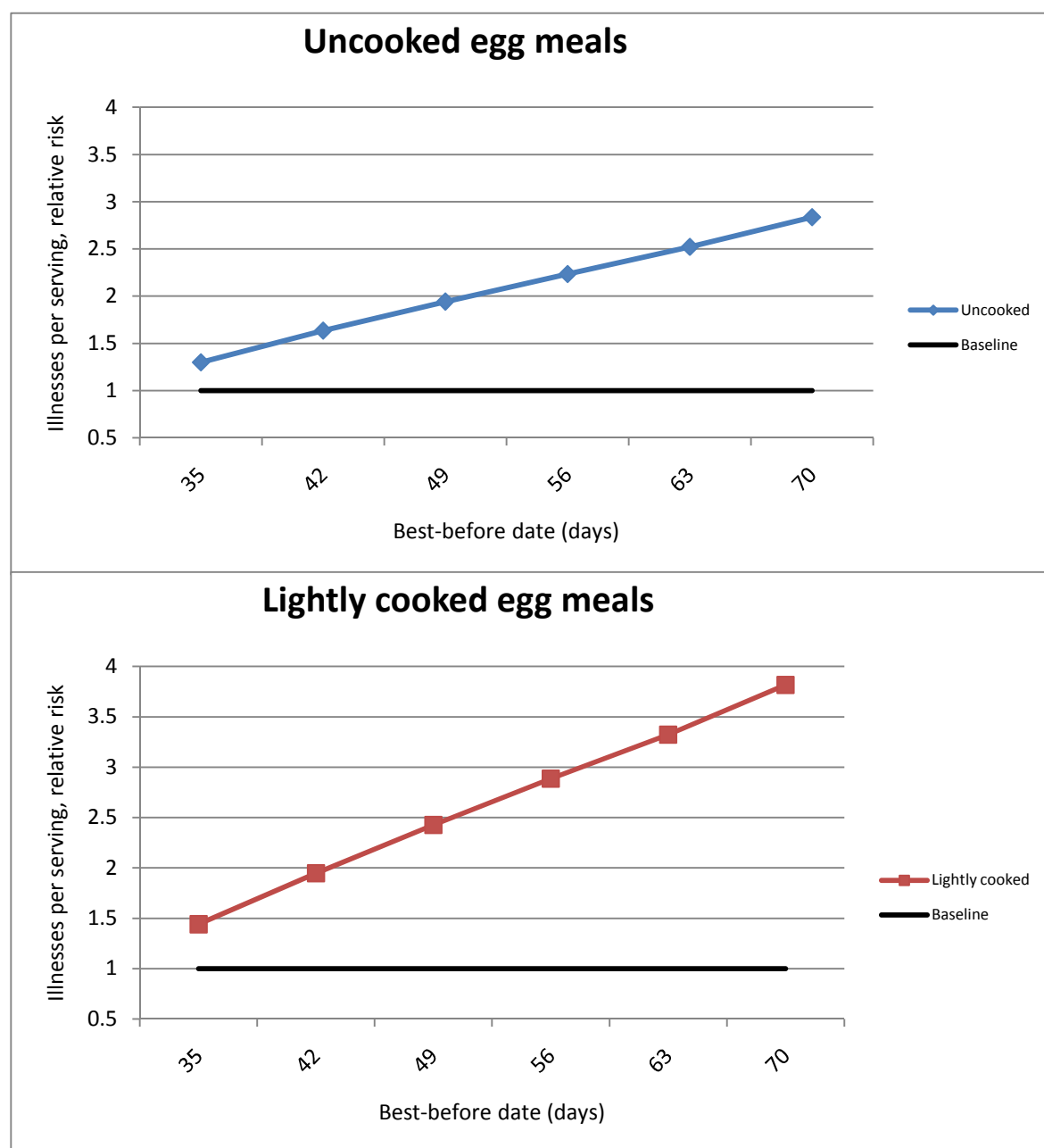


Figure 21: Relative risk of illness from uncooked and lightly cooked egg meals under alternative storage conditions in catering establishments different from those of the baseline scenario (i.e. current egg storage conditions). The risk was calculated as the mean number of illnesses per serving

13.3.3.1. Pooling - catering/food service and institutional settings

The results from the EPM for pooled eggs used in catering/food service and institutional settings are similar to those obtained when pooling takes place in the household. The baseline scenario (i.e. sell-by date of 21 days and best-before date of 28) was compared to a scenario of extending the best-before date to 70 days in weekly intervals for uncooked and lightly cooked meals. The relative risk of illness estimates show an increase of the risk with storage time, similar to that observed on individual eggs (Figure 22).

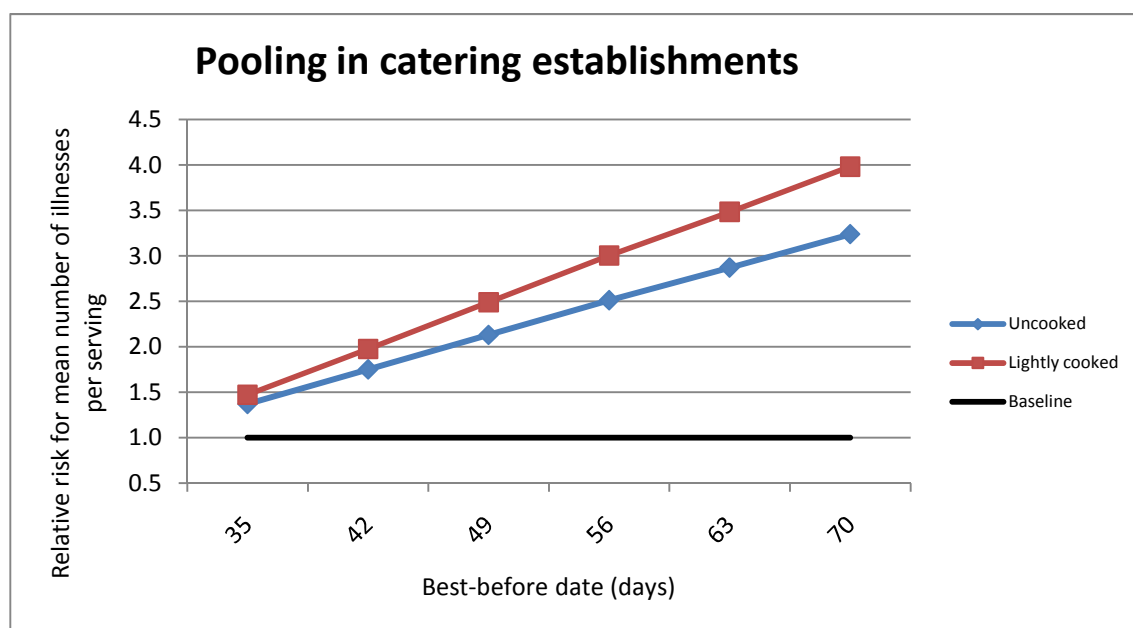


Figure 22: Relative risk of illness from uncooked and lightly cooked egg meals made with pooled eggs in catering establishments, under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions). The risk was calculated as the mean number of illnesses per serving

As with pooling at the household, to account for the increased probability of illness for consumers of servings derived from the contaminated pool, the risk was also expressed as the probability of at least one of the consumers exposed to the contaminated pool servings becoming sick. For pooling at catering establishments, the number of exposed individuals was set at 50, the same as the most likely number of eggs used in the pool. The probability of at least 1 of the 50 people getting sick arising from consumption of uncooked dishes rises from 0.91 under baseline conditions to between 0.96 to 1.00 depending on the prolongation of the best-before date. For lightly cooked eggs the probability changes from 0.76 to between 0.88 and 1.00) (Figure 25).

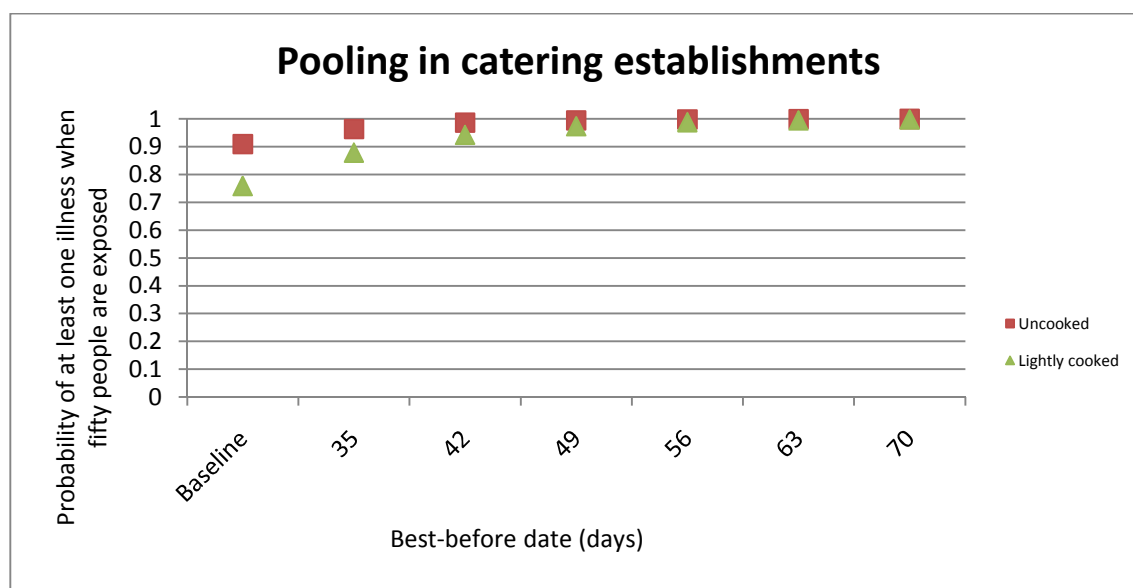


Figure 23: Probability of at least one illness when fifty people are exposed to uncooked or lightly cooked egg meals made with pooled eggs in catering establishments, under alternative storage conditions different from those of the baseline scenario (i.e. current egg storage conditions)

13.4. Uncertainty evaluation

13.4.1. Background

In the EFSA context, the term ‘uncertainty’ is intended to cover ‘*all types of limitations in knowledge, at the time it is collected*’ in the risk assessment process (EFSA, 2009a). The need to address uncertainty is expressed in the Codex Working Principles for Risk Analysis. These state that ‘*constraints, uncertainties and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner*’ (CODEX, 2007). The Scientific Committee of EFSA explicitly endorsed this principle in its guidance on transparency in risk assessment (EFSA, 2009a). Therefore it is recognised that in the risk assessment process it is important to characterise, document and explain all types of uncertainty arising in the process.

Ideally the analysis of the uncertainty in a risk assessment would require the following steps:

- identifying uncertainties;
- describing uncertainties;
- evaluating uncertainties around individual factors in their own scales;
- evaluating the impact of individual factors uncertainties on the assessment outcome;
- evaluating the combined impact of multiple uncertainties on the assessment outcome including evaluating how much the combined uncertainties downgrade the weight of the evidence.

The last three steps can be conducted at three levels: qualitative; deterministic; and probabilistic.

The EFSA Working Group on Uncertainty in Risk Assessment²⁹ is currently formulating guidelines on how the uncertainty analysis should be performed in a harmonised and structured way. As this is still on-going, this opinion addresses only the first two steps, i.e. identification and description of the uncertainty.

13.4.2. Identification and description of the sources of uncertainty

The MAESM and EPM models used to assess the potential change in the public health risk arising from the prolonged egg shelf life rely on three main sets of assumptions that include:

- the way the growth of *S. Enteritidis* takes place (i.e. which environmental factors to consider and how they affect the microbiological process);
- the stages and practices that are considered relevant in the specific risk assessment context;
- the shape and the parameters of the distributions used to account for the variability of the factors affecting the growth of *S. Enteritidis*.

The first two categories of assumptions can be considered sources of uncertainty that can influence the direction and size of the effect of prolonging the storage of eggs on the risk of salmonellosis either in isolation or in combination. The third one is in principle related to the need to account for the variability in the phenomenon (e.g. variability in the practice of time and temperature storage in the various MSs, variability in the reduction of the *S. Enteritidis* organisms due to cooking etc.). Owing to a lack of sound data on the true distribution of the relevant factors that are involved in the model, the above-mentioned distributions have to be considered as expert ‘best guesses’ based on the available information. Therefore they do not only account for variability but they also represent sources of uncertainty.

²⁹ See www.efsa.europa.eu/en/scer/scerwgs.htm for details

Tables 16 and Table 17 list the potential sources of uncertainties as related to the three sets of assumptions and provide a narrative description of the direction of the impact that these uncertainties could have on the final outcome for the MAESM and EPM respectively.

Table 16: Potential sources of uncertainties arising from the MAESM assumptions and qualitative assessment of the impact that these uncertainties could have on the final outcome

Outcome	Source of uncertainty	Direction of the effect on the outcome
Prevalence of <i>S. Enteritidis</i> contaminated eggs	Sensitivity and specificity of the sampling and testing methodologies for estimates of <i>S. Enteritidis</i> flock prevalence	It is expected that the sensitivity and specificity of the sampling and testing methodologies are not perfect, so the prevalence might be underestimated if the sensitivity is lower than 100 %. This will affect only absolute measures of risk and not relative risk estimates, as they remain unchanged from the baseline to the alternative scenarios assessed.
	Use of data from the United Kingdom to estimate within-flock prevalence and the rate of contaminated eggs laid by infected hens	It is assumed that flocks and hens in the EU would have similar rates as in the UK. The flock <i>S. Enteritidis</i> and Typhimurium prevalence reported by the baseline survey and by the harmonised monitoring programme is lower than the EU average. There is little data on within-flock prevalence across the EU, but in UK vaccination for <i>S. Enteritidis</i> is used for laying flocks, which is likely to reduce the within-flock prevalence. This means that the absolute risk estimates in Section 13.3 could be underestimated. This will affect only absolute measures of risk and not relative risk estimates, as they remain unchanged from the baseline to the alternative scenarios assessed.
Growth of <i>S. Enteritidis</i> in a contaminated egg	Lack of sound data on the distribution of: -initial and maximum number of <i>S. Enteritidis</i> in a contaminated egg -storage time and temperature in the various stages -reduction in the number of organisms due to cooking practices	Due to the scarcity of data it is difficult to predict what is the direction and size of the impact of the uncertainty on the single variable distribution. Initial and maximum number of organisms in one egg and storage temperature and time are positively correlated with the estimated number of <i>S. Enteritidis</i> in contaminated eggs. Therefore their impact on the estimated number of organisms goes in the same direction as the bias that affects the parameters of their distribution. The reduction due to cooking are negatively correlated with the number of organisms per portion (due to the mitigation effect). Therefore its impact on this outcome goes in the opposite direction to the bias that affects the parameters of their distribution. Although these uncertainties influence the overall risk, they have no effect on the relative risk estimates in this Opinion, as they are constant in the various scenarios.
	Only internal contamination of eggs is considered	Although the occurrence of <i>S. Enteritidis</i> could be underestimated as a result of not considering contamination on the shell, the impact on the change in the risk due to prolonging the shelf life is expected to be negligible because shell contamination is not expected to increase as a result prolonging the storage time. Therefore this uncertainty is not expected to affect the relative risk.

Outcome	Source of uncertainty	Direction of the effect on the outcome
	Growth only starts only when the yolk membrane has deteriorated	The effect of this assumption could be an underestimation of the number of <i>S. Enteritidis</i> organisms at the end of the process for the cases in which the growth started before the degradation of the yolk (i.e. in the albumen). On the other hand, the mathematical model used to predict the YMT likely overestimates the proportion of eggs able to support growth, as it predicts the time when degradation occurs in 20 % of the eggs. Overall this source of uncertainty is not expected to affect the change in the relative risk, as the same assumption is made for the various scenarios.
	Use of the Rosso equation on the basis of the distribution of the temperature at each stage	The equation depends on the minimum, maximum and optimal temperatures for growth, which are based on previous estimates. Their impact on growth could go in different directions. It also depends on the expected temperature at each stage. The more this temperature is under- (over-) estimated the more the growth is biased in the same direction. Overall this source of uncertainty is not expected to affect the change in the relative risk, as the same assumption is made for the various scenarios.
Storage time at retail/household	It is assumed that most eggs do not stay at retail/household up to the sell-by/best-before date but are sold/consumed in the early part of the stage (see paragraph 13.2).	The distributional assumption is not conservative in the sense that it gives higher probability to a low storage time at retail/household level. Nonetheless it is quite realistic since for commercial and safety reasons retail establishments /households are interested in selling/consuming the eggs as soon as possible. If the assumption is over-optimistic (i.e. underestimate the retail/household storage time) the mean number of organisms per serving at the end of the process might be underestimated and so the probability of illness.
Probability of illness per infected serving	D2TAP dose-response model: -Estimated on the basis of all serovars (not only <i>S. Enteritidis</i>) -Vulnerable people have the same probability of illness as the overall population -Recourse to Beta-Poisson model towards a re-parameterised Weibull -The serving size was set as a single egg	Although not confirmed by analysis of currently available epidemiological data, it is possible that the probability of illness for vulnerable people is underestimated. Again, given that the same dose-response model is used in the baseline and the alternative scenarios, this uncertainty is not expected to affect the relative risk estimates. The risk to consumers of contaminated eggs will depend on the quantity of egg or serving consumed, with greater risk associated to bigger servings. If the choice of serving size for this assessment is smaller than the average serving size in the EU, the risk will be underestimated (and if it is bigger, the risk will be smaller). This source of uncertainty is not expected to affect the change in the relative risk since the same assumption is made for the various scenarios.

Table 17: Potential sources of uncertainties arising from assumptions in the EPM and qualitative assessment of the impact that these uncertainties could have on the final outcome

Outcome	Source of uncertainty	Direction of the effect on the outcome
Initial number of <i>S. Enteritidis</i> in a pool	It is assumed that only one egg is contaminated	This assumption is not conservative since it will underestimate the number of organisms in the pool at the time of pooling. However, the probability that more than one egg is contaminated in a pool - considering the estimated prevalence in EU of 4/10 000 – is 20/1 000 for 50 eggs/pool (most likely value for catering and food service and institutional settings) and 2.4/1 000 for 6 eggs/pool (most likely value for home).
Growth of <i>S. Enteritidis</i> in a contaminated pool	Lack of sound data on the distribution of: -number of eggs used for pooling, both at household level and in catering establishments -storage time and temperature after pooling Maximum contamination in a pool only depends on the quantity of material available The temperature of the egg pool corresponds to the storage temperature	Due to the scarcity of data it is difficult to predict what is the direction and size of the impact of the uncertainty on the single variable distribution. Initial and maximum number of organisms in one egg and storage temperature and time are positively correlated to the estimated number of <i>S. Enteritidis</i> in contaminated eggs at the end of storage. No evidence is available on how different food substrates in pooled egg meals can favour growth of <i>S. Enteritidis</i> . Therefore it is difficult to predict what is the direction and size of the impact of the uncertainty on the single variable distribution. This assumption is conservative when the storage temperature is above the temperature of egg pool (most likely in the case of ambient temperature) and vice-versa (most likely in the case of refrigeration). Since the pooling module is applied to the worst case scenario (ambient temperature) the approach is most likely to be conservative.
Number of <i>S. Enteritidis</i> per serving	Content of <i>S. Enteritidis</i> is equal for each serving obtained from the same pool	Contamination is usually not uniform as <i>Salmonella</i> occurs as micro colonies –no single uniformly distributed organisms– and attaches to particles, so even with homogenisation it is difficult to achieve true uniformity except in a liquid. The potential impact of this assumption would be larger in the case of larger pool sizes. If the assumption is not correct, the dilution effect does not apply evenly. Therefore the content of <i>S. Enteritidis</i> might be overestimated in some servings and underestimated in others. For pools containing a very high number of <i>S. Enteritidis</i> organisms the impact could be limited, since a level capable of inducing illness is likely to be reached in any serving. The same would not apply to low/medium contaminated pools in which the organisms could concentrate in a limited number of servings. In these cases, the risk estimates might be overestimated, as some servings will be more contaminated but others will not, so less people will become ill.
Number of eggs per serving	The serving size consumed from egg pools is set as the equivalent amount to a single egg.	Similar considerations as in Table 16 apply.

As explained in these tables, the uncertainties associated to the assumptions made and to the data used in this assessment will affect the absolute estimates of the risk. The combined effect of all the uncertainties is difficult to measure, but nevertheless the absolute estimates in Section 13.3 should be used with caution. The relative risk estimates are less influenced by uncertainty associated to both the baseline and the alternative scenarios.

Concluding remarks

- In order to answer ToR1, the Panel decided to use a quantitative model. Following the analysis of the available models, a modified version of the Australian Egg Corporation Limited model was adopted since it was the one that best suited the needs of this opinion.
- This model, denoted as MAESM, estimates the effect of storage time and temperature on the ability of *S. Enteritidis* to grow in egg contents. All stages before lay are excluded, since it is considered that possible changes in the eggs' shelf life will impact the behaviour of *S. Enteritidis* only from the point of lay onward, as all previous steps will remain unchanged.
- The original AECL model was modified to ensure that it was fit for purpose and to include data relevant to the EU situation, as follows: 1) the prevalence of contaminated eggs was updated to include European data on *S. Enteritidis*, instead of non-*S. Enteritidis* serovars; 2) the number of *S. Enteritidis* present in the egg at the time of lay and during the first 24 hours after lay was adjusted; 3) ambient storage temperatures and times for on-farm, transportation and processing stages relevant to the EU situation were added; 4) the stages for transportation from retail to household and household storage were added to the model; 5) the proportion of eggs refrigerated during retail and household storage was added to the model; 6) the maximum specific growth rate at optimum temperature used in the AECL model was modified.
- In addition, a module representing pooling practices, denoted as EPM, was developed to assess the impact of the use of eggs under prolonged storage conditions for products that require pooling, both in household and catering/food service and institutional settings. The impact of pooling on the prevalence of contaminated servings was not investigated, as it is not as influenced by prolonged egg storage as the growth of *S. Enteritidis*.
- Only *S. Enteritidis* internal contamination was considered, as the risk from the presence of *Salmonella* on the shells does not increase as a result of a prolongation of storage time. *Salmonella* possibly present on the shell will not grow during storage and are more likely to decline in number.
- The actual situation (baseline scenario) was compared with various alternative scenarios. The storage conditions were the following:
 - Baseline scenario: sell-by-date 21 days after lay, best-before-date 28 days after lay;
 - Alternative scenario at household: the best-before date was extended to a maximum of 70 days post-lay. The current sell-by date of 21 days was replaced by 28, 35 and 42 days. The temperatures used at retail for these alternative scenarios will be a) the same as in the baseline, and b) at refrigeration-only temperature;
 - Alternative scenario at catering/food service and institutional settings: the best-before date was equally extended to a maximum of 70 days post-lay.
- According to the MAESM model results, a prolongation of the storage time for table eggs is expected to result in an increase of the number of illnesses per million servings, except when eggs are well-cooked. The magnitude of this increase depends on the additional time of storage that the eggs spend at both retail and households. The only way to minimise any

increase in risk during extended storage is to keep the eggs refrigerated both at retail and the household.

- For example, extending only the sell-by date by one week (from 21 to 28 days) is estimated to result 61 and 45 salmonellosis cases per million servings for uncooked and lightly cooked egg meals respectively, equivalent to an increase of 18 and 15 cases compared to the baseline, and a relative risk of illness of 1.4 and 1.5. If in addition the best-before date is extended by one week (from 28 to 35 days), the number of cases per million servings for uncooked and lightly cooked egg meals would be 68 and 51, with a relative risk of 1.6 and 1.7. In the worst case scenario considered in this assessment (sell-by date of 42 days, best before date of 70 days), such figures would be 127 and 104 (84 and 74 additional cases for uncooked and lightly cooked egg meals compared to the baseline). The relative risk would be: 2.9 and 3.5.
- The implementation of refrigeration as currently used in the EU (with temperatures assumed to range from 0 to 12 °C) during the retail stage limits to some extent this increase in the public health risk, but in most cases the resulting risk is still greater than the estimate for the baseline situation. The risk is reduced in the case of a prolongation of up to three weeks in the sell-by date, and also of one or two weeks for the best-before date for a sell-by date of 35 and 28 days respectively. If refrigeration is applied during storage in all retail establishments, the risk is reduced when compared with the current situation, but if the sell-by date or the best-before date are prolonged beyond three weeks, the risk estimates are greater, even if refrigeration at retail is applied.
- Similarly to results obtained considering household consumption, a prolongation of storage time of eggs used in catering/food service and institutional settings would lead to an increase in the risk of salmonellosis. The calculated mean number of illnesses would increase from 24 (uncooked egg meals) and 13 (lightly cooked egg meals) cases of illness per million serving in the baseline scenario, to 32 and 19 cases of salmonellosis with a prolongation of one week of the best-before date, respectively. The calculated relative risk of illnesses would be 1.3 (uncooked egg meals) and 1.4 (lightly cooked egg meals), when compared to the baseline scenario.
- For pooling of eggs, the relative risk of illness estimates show a similar increase of the risk with storage time to that observed on individual eggs.
- The uncertainties associated to the assumptions made and to the data used in this assessment will affect the absolute estimates of the risk. The combined effect of all the uncertainties is difficult to measure, but nevertheless the absolute estimates in Section 13.3 should be used with caution. The relative risk estimates are less influenced by uncertainty associated to both the baseline and the alternative scenarios.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

ToR1

Assess the public health risk posed by relevant pathogens and in particular by *Salmonella* in the consumption and handling of table eggs and to quantify the relevance of underlying factors such as the observed flock prevalence in a Member State/region, the period of time between laying and consumption, the cooling and storage conditions, consumer behaviour (e.g. cooking, cross-contamination) etc.

- *Salmonella* spp. and *S. Enteritidis* accounted for more than 85 % and 65 % respectively of strong evidence outbreaks caused by eggs and egg products in the EU in 2012, according to official data.
- *Salmonella* serovars differ in their ability to cause contamination on the eggshell or in egg contents, with *S. Enteritidis* causing a higher rate of contamination of egg contents and a lower rate of contamination of eggshells compared with other serovars.
- The application of *Salmonella* control programmes at EU level since 2007 has led to a decrease in the incidence of *S. Enteritidis* infection in laying hen flocks, and in the same period the human infections due to *S. Enteritidis* have also decreased.
- To assess the impact of a prolongation of the shelf-life of table eggs on the number of salmonellosis cases in the EU, a mathematical model was used. The sell-by date was increased from the current 21 days to 28, 35 and 42 days, up to a total of 70 days of storage from lay to consumption (i.e. the best-before date). By the prolongation of the storage time, these distributions retain their shape, but shift to the right, reflecting that the behavioural patterns of retailers and consumers are assumed to remain the same if the sell-by date or best-before date are extended. Meals were either uncooked, lightly cooked or well-cooked eggs.
- According to the model results, a prolongation of the storage time for table eggs results in an increase of the number of illnesses per million servings, except when eggs are well-cooked. The magnitude of this increase depends on the additional time of storage that the eggs spend at both retail and households. An effective way to minimise any increase in risk during extended storage is to keep the eggs refrigerated both at retail and the household.
- Among different scenarios explored by the model, extending only the sell-by date by one week (from 21 to 28 days) is estimated to result in a relative risk of illness of 1.4 and 1.5 for uncooked and lightly cooked egg meals respectively, when compared to the baseline. If the best-before date is also extended by one week (from 28 to 35 days), the relative risk would be 1.6 and 1.7. In the worst case scenario considered in this assessment (sell-by date of 42 days, best before date of 70 days), such figures would be 2.9 and 3.5. It should be noted that the absolute risk is greater for uncooked meals compared to lightly cooked meals.
- The implementation of refrigeration as currently used in the EU (with temperatures assumed to range from 0 to 12 °C) during the retail stage reduces to some extent this increase in the risk, but in some scenarios the resulting risk is greater than the estimate for the current situation (i.e. with an assumed most likely 24 % of consumers continuing to store the eggs at room temperature). Specifically, the risk is lower when compared with the current situation in the case of a prolongation of up to three weeks in the sell-by date, and also of one or two weeks of the best-before date, if refrigeration is applied during storage in all retail establishments. If the sell-by date or the best-before date are prolonged beyond three weeks, the risk estimates are greater, even if refrigeration at retail is applied.

- Similarly to results obtained considering household consumption, a prolongation of storage time of eggs used in catering/food service and institutional settings would lead to an increase in the risk of salmonellosis. The calculated relative risk of illnesses would be 1.3 (uncooked egg meals) and 1.4 (lightly cooked egg meals), when compared to the baseline scenario, with a prolongation of one week of the best-before date. It should be noted, however, that the absolute risk is greater for uncooked meals compared to lightly cooked meals.
- For pooling of eggs, the relative risk of illness estimates show a similar increase of the risk with storage time to that observed on individual eggs.
- The uncertainties associated to the assumptions made and to the data used in this assessment will affect the absolute estimates of the risk. The combined effect of all the uncertainties is difficult to measure, but nevertheless the absolute risk estimates should be used with caution. The relative risk estimates are less influenced by uncertainty associated to both the baseline and the alternative scenarios.
- According to EU outbreak data, non-*Salmonella* egg-borne illnesses have been associated with *Staphylococcus aureus* and *Bacillus cereus* group toxins in the EU in 2012. Other microorganisms can be found on or in eggs, but eggs are not a significant vehicle for foodborne disease other than for *Salmonella*.
- The impact of a prolongation of the storage time on the risk posed by these pathogens is currently unclear, but nevertheless the contribution of eggs and egg products to human illnesses caused by these pathogens is likely to remain minor in comparison with other foodborne and non-foodborne sources.
- The relationship between environmental temperature, relative humidity and eggshell temperature affects the development of condensation. Cold chain disruption is one factor increasing the risk of condensation and this could increase bacterial penetration into the egg.
- There is little information at the EU level on the production and consumption of eggs other than eggs from *Gallus gallus*.
- There are no formal *Salmonella* control programs applied in the EU in species other than *Gallus gallus*, and infection may be common in some species, such as ducks.
- The role of *S. Enteritidis* in these species is not as prominent as in *Gallus gallus*, but *S. Typhimurium* appears relevant in ducks and has been reported to cause duck-related egg-borne outbreaks.
- There are insufficient data to assess the effect of increasing storage time of eggs from species other than *Gallus gallus* on the public health risk derived from consumption of eggs.

ToR2

Assess the public health risk deriving from deterioration taking into account the underlying risk factors such as hygiene, the cooling and storage conditions in the period between laying and consumption, consumer behaviour, etc.

- At the time of lay, the risk of internalisation of contamination into the eggs is minimal due to the integrity of the cumulative physical barriers of the cuticle, the eggshell, the shell membranes, the chalazae and the vitelline membrane, and to the efficacy of the antimicrobial activities of egg albumen.

- During storage, gaseous exchanges between the egg content and the atmosphere, as well as exchanges of water and minerals between egg albumen and egg yolk lead to decreasing egg albumen defence mechanisms and weakening of the vitelline membrane, increasing the risk for bacterial invasion of the egg internal compartments.
- There is a clear deleterious effect of high storage temperatures and/or long storage periods on the internal egg quality and the rate of development of macroscopic changes in table eggs, particularly if eggs are contaminated by spoilage bacteria. While the effect of the storage temperature on the level of surface bacteria is variable according to a combination of conditions; temperature, time, and humidity are crucial parameters involved in the decrease of egg quality throughout storage, increasing the risk of microbial invasion of the egg. Storage at chilled temperatures helps maintain overall physicochemical and microbiological quality of eggs.
- Egg spoilage events strongly depend on the hygienic conditions of egg production and practices of egg handling, including storage times and temperatures.
- At ambient temperature, there is a lag period of around 10 to 20 days before detection of appreciable numbers of trans-shell penetrated spoilage bacteria in the egg content, and before the appearance of macroscopic changes in the egg content.
- Gram-positive genera such as *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Aerococcus*, and, to a lesser extent, *Bacillus* dominate on the shell surface. Gram-negative genera, such as *Escherichia*, *Alcaligenes*, *Proteus*, *Flavobacterium* and *Pseudomonas*, are minor surface contaminants. However, these have a better ability to penetrate eggs than other bacterial genera, resulting in contamination and, eventually, spoilage. On some occasions, moulds of the genera *Penicilium*, *Alternaria*, and *Mucor* can also spoil eggs.
- The characteristics of egg spoilage are mainly the results of macroscopic changes in their odour and/or colour or viscosity, which would prevent the egg being used for food products.

ToR3

Assess possible consequences for public health of an extended shelf-life (after the sell-by date) of table eggs for the specific freshness criteria for egg products as laid out in the hygiene package.

- There is little data on the quality indicators for the specific freshness criteria for egg products as laid out in the hygiene package, which prevents a quantitative assessment of the evolution of the concentration of these indicators with storage time.
- In those countries where egg products are not derived from retail table eggs that have reached the end of their shelf life, a prolongation of the storage time of these eggs will not influence the levels of these indicators in egg products.
- The levels of lactic acid found in eggs that are past their shelf life are less than those found in some other food products, such as fermented milk products (e.g. yogurt or cheese).
- Regarding egg product processing, the control of the physicochemical and microbial quality of the raw material, the strict control of the pasteurisation step, as well as the refrigeration of pasteurised liquid egg products at a strictly constant temperature of 4 °C or lower are currently the only effective ways to control microbial contamination in pasteurised liquid egg products.
- In general, chemical indicators of egg deterioration increase with time and temperature, but studies of this in the scientific literature report variable results.

- Of the two currently-recommended indicators, 3-hydroxybutyric acid is exclusively related to detection of the use of embryonated eggs, and is therefore related more to fraudulent practice than to microbial growth or the conditions of storage as its concentration is not influenced by storage time if eggs are not embryonated. Even if present at trace levels in infertile eggs, its concentration does not increase during the storage, regardless of the storage conditions.
- Lactic acid is recognised as an indicator of microbial degradation of table eggs. It is present in the egg due both to the development of the embryo in fertile eggs and to microbial growth. The latter will be affected by the conditions of storage with the concentration of lactic acid increasing with egg storage time.
- Microbiological criteria are set in European legislation for egg products, i.e. a food safety criterion for *Salmonella* and a process hygiene criterion for Enterobacteriaceae at the end of the process of egg product manufacturing and these provide a suitable indication of microbial contamination.

RECOMMENDATIONS

- Additional studies on the potential for growth of *Salmonella* in relation to the breakdown of the yolk membrane are necessary, particularly under more realistic conditions (e.g. *Salmonella* contamination levels that are found in naturally contaminated eggs from large scale production systems) than those conducted to date. These studies should also be conducted in eggs from species other than *Gallus gallus*.
- This Opinion focused mainly on the effect of time of storage on public health. There is a need for additional risk assessments to be conducted, exploring the effect of different temperatures of storage of eggs, from the point of lay to consumption, on the risk posed by egg borne pathogens such as *S. Enteritidis*.
- Further studies to investigate the occurrence and control of microorganisms during industrial production of egg products, including pasteurisation, if the storage of eggs is prolonged.
- The use of poor quality eggs for which biochemical spoilage indicators were selected is no longer common in current egg processing. The relevance of current indicators should therefore be evaluated, and the possibility of using more relevant indicators considered.
- Data on consumption of eggs and egg products (e.g. cooking practices, serving sizes, etc) are necessary to further refine the risk estimates in this Opinion.
- Collection of data on the production, use and consumption of eggs from species other than *Gallus gallus* to assess the public health risk arising from the consumption of eggs, from these species in the EU.
- Further studies to assess the effect of increasing storage time of eggs from species other than *Gallus gallus* on the public health risk derived from consumption of eggs, to obtain more data on the structure of the eggs from these species e.g. shell permeability, inhibitory properties of the albumen, dynamics of membrane breakdown, multiplication of *Salmonella*; as well as the frequency of shell and internal egg contamination by relevant hazards.

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APPENDICES

Appendix A. Microbial and physicochemical quality of egg products

Spoilage of egg products

The process of egg product manufacturing systematically induces egg content contamination because of the unavoidable contact with the contaminated eggshells at the breaking step. Moreover, once the physical barriers of the shell and the eggshell membranes removed, the egg loses most of its natural antimicrobial defences. The processes of separation and stabilization have crucial effects on the subsequent spoilage events, particularly when the egg products are used in susceptible foods such as refrigerated desserts.

Egg yolk and whole egg are of key concern since they are more prone to spoilage than egg white, the latter retaining at least partly its antimicrobial properties. The flora of raw liquid egg products generally comprises the gram-negative bacteria potentially involved in the spoilage of table eggs. Nowadays, there is a low occurrence of events of spoilage of raw egg products if appropriate storage conditions are respected prior to pasteurization, i.e. at a temperature not exceeding 4 °C for no more than a few hours. After pasteurization, due to the low levels of the heat treatments applied in the sector of egg product manufacturing, it is generally recognized around 1 % of bacteria survive. The surviving bacteria are gram-positive cells of the genera *Streptococcus*, *Enterococcus* and, as expected, the spore-forming *Bacillus*. With the exception of (Miller et al., 2010), who discussed the expression of proteolytic and lipolytic activities by *Enterococcus* spp. Strains isolated from whole egg products, the involvement of bacterial enzymatic activities in the spoilage of egg products has not received sufficient attention for establishing a clear relationship between a type of bacterium and a specific characteristic of spoilage. Moreover, the putative involvement of heat-resisting enzymes in spoilage events, well-known in the dairy industry, has never been investigated in the egg product environment. However, *Bacillus* species, and particularly those belonging to the *B. cereus* group, appear as one of the main flora involved in spoilage events in the sector of egg product processing, leading to heavy economic losses (Baron et al., 2007; Jan et al., 2011). These bacteria are known to express various enzymatic activities (lipases, proteases and phospholipases) responsible for food spoilage, even at refrigerated temperatures when psychrotrophic strains are involved. They are also recognized as particularly resisting the industrial processes, due to their ability to adhere on industrial stainless steel surfaces and to persist on and in equipment in the form of biofilms. Moreover, the heat treatments themselves may lead to spore germination and multiplication in the pasteurized egg product, as already demonstrated for pasteurized dairy products (Meer et al., 1991). Even if *B. cereus* group bacteria are present at a low levels on the eggshell surface and in raw egg (Protais et al., 2006; Koné et al., 2013), the heat destruction of their vegetative competitors may facilitate their development in the pasteurized egg product.

The contamination by the *B. cereus* group bacteria is thus of particular concern for the egg product sector, particularly for chilled liquid egg products, where shelf-lives range from a few weeks to three months. Storage at refrigerated temperature may lead to the selection of psychrotrophic strains of the group. In addition to these economic problems, the question of health issues can not be neglected since a psychrotolerant *B. weihenstephanensis* strain, isolated from a spoiled liquid egg product, has already been shown to produce toxins (Baron et al., 2007).

Other events of egg product spoilage are mainly due to gram-negative bacteria, such as *Pseudomonas* and *Enterobacteriaceae*, generally arising from inadequate heating or post-pasteurisation contaminations. These events can be controlled by correcting the pasteurisation protocols and/or by improving the sanitation processes.

The bacterial spoilage of liquid egg products can lead to technological and/or sensorial problems (coagulation, modification of the colour and/or the flavour) due to the production of bacterial hydrolytic enzymes, even at low temperature.

For liquid whole egg and egg yolk, spoilage often involves visible modifications such as coagulation and/or colour changes. The other spoilage characteristics are changes in the consistency, the viscosity or the flavour. These egg products are particularly rich in proteins and lipids, including phospholipids. The main enzymes involved in their spoilage are the lipases and proteases expressed by *Enterococcus* spp. and by the *B. cereus* group. The lecithinase activity of the *B. cereus* group may also lead to the destabilization of the binding properties of egg yolk, inducing marked modifications in the colloidal state of the egg components.

Considering liquid egg white, the visual detection of spoilage is not obvious. A phenomenon of liquefaction may occur throughout storage time, mainly due to protein denaturation accompanying bacterial growth. Nevertheless, considering the well-known antimicrobial properties of egg white, this egg product does not favour bacterial growth, contrary to egg yolk and whole egg which are highly nutritional media. Events of egg white spoilage mainly imply defects in the breaking process, providing nutrients from the egg yolk in the case of poor separation of the different egg compartments.

Dried egg products are rarely affected by spoilage because of their low water availability. If events of spoilage occur, they are probably due to mishandling enhancing the water activity (a_w). In frozen egg products, microbial growth is also prevented by low water availability. Processed egg products such as hard-boiled, scrambled and toaster eggs are generally cooked under temperatures higher than 71 °C, the process involving the coagulation of egg proteins. These temperatures kill the vegetative forms of spoilage microorganisms. Moreover, these foods are often sold frozen, avoiding subsequent microbial growth.

Physicochemical quality of egg products

The functional quality of the egg, based on its emulsifying, foaming, gelling, thickening, colouring and aromatic properties, is widely used in domestic kitchen and in the food industry. Whole egg is widely used since it combines these functional qualities. For specific applications, egg white and egg yolk may be used separately. While egg white is a key ingredient in terms of foaming, egg yolk is widely used for its emulsifying, aromatic and colouring properties.

2.1. Effect of storage on foaming properties

The foaming properties of egg white depend on the quality of the raw material. It is frequently observed that there is an inverse relationship between the foaming capacity and the stability of the foam. Modifications in pH, the viscosity, the hydrophobicity of proteins and the content of disulfide bounds are supposed to increase the foaming capacity while decreasing the stability of the foam (Nakamura and Sato, 1964; Hammershoj and Larsen, 1999; Hammershoj et al., 2004).

The egg white viscosity depends on numerous factors, including the storage conditions and the compartment considered (thin or thick albumen) (Lechevalier et al., 2010). Other things being equal, the viscosity of the thick albumen is 40 times higher than that of the thin albumen (Lang and Rha, 1982). It decreases with increasing length and temperature of storage (Sauveur, 1988; Lucisano et al., 1996), due to the dissociation of the ovomucin-lysozyme complex, either due to pH increase and dissociation of the electrostatic interactions, or to the denaturation of one or both proteins (Hawthorne, 1950; Sato et al., 1976).

The thick albumen exhibits a foaming capacity around 30 % lower than the one of the thin albumen. However, the stability of the foam is around 30 % superior (Nakamura and Sato, 1964). These differences are less obvious when the egg ages, since the foaming capacity of the thick albumen tends to approach that of the thin albumen during storage (Nau et al., 1996). However, the effect of storage on the foaming capacity of egg white appears contradictory in the literature. Yang and Baldwin (1995) and Nau et al. (1996), considered that the storage had a positive effect on the foaming capacity. Other authors have shown the absence of effect of storage on this property (Makhlouf et al., 1983; Ikeme, 1987; Hammershoj and Qvist, 2001). The same contradictory effect of storage is highlighted on the

stability of the egg white foam. A decrease was observed by Nau et al. (1996), an increase by Hammershoj and Qvist (2001) and no effect by Makhoul et al. (1983) and Ikeme (1987).

Considering the effect of the storage temperature, Silversides and Budgell (2004) showed an increase in the egg white foaming properties (whipping volume) after 10 days at 21 °C, whereas a decrease was observed by Jones (2007) after shell egg storage for 8 weeks at 4 °C. Hammershoj and Qvist (2001) observed that the stability of the thick albumen foam was at its maximum after a storage period of 20 to 40 days at 4 °C.

The freshness of the egg and the quality of the vitelline membrane are other crucial parameters influencing the foaming capacities of egg white. The better the membrane resistance, the better the separation between egg white and egg yolk. Even present as trace levels (0.1 to 0.2 %), egg yolk strongly decreases the foaming capacity of egg white (St. John and Flor, 1930; Lechevalier et al., 2005). The addition of 5 to 20 % egg white in egg yolk also leads to a decrease in egg yolk viscosity in the range of 6-90 % (Li-Chan et al., 1995). The resistance of the vitelline membrane is decreased when eggs are submitted to vibrations during transport and storage, and especially at high temperature (Berardinelli et al., 2003; Chen et al., 2005). According to Lechevalier et al. (2005), 70 % of the variability of the foaming properties of pasteurised egg white is due to changes in storage temperatures of table eggs upstream of the breaking process.

2.2. Effect of storage on the gelling properties of egg white

The gelling properties of egg white are maximal after around 14 days of shell egg storage at 4 °C, a time at which pH is alkaline (around 9) and the concentration of S-ovalbumin is weak (Lechevalier et al., 2011). Alkaline pH increases the strength of the heat-induced egg white gels and also the capacity of the gels to retain water (Hickson et al., 1982). When heated at pH 9, the egg white gel network is denser, more uniform and contains fewer pores and smaller protein particles than at pH 7. Handa et al. (1998) have shown that the disulfide bonds increased in the pH range 7-9 and increase the formation of the egg white gel network and its capacity to retain water.

2.3. Effect of storage on egg flavour

Egg storage may affect the flavour of the egg content. According to Koehler and Jacobson (1972), sulphurous and chemical flavours, as well as astringency, increase while the sweet flavour decreases with the storage temperature, in the range 0 to 22 °C and with the storage period, in the range 0 to 18 weeks. Fish odours appear in egg white from 10 weeks at 22 °C. Under accelerated aging conditions (32 °C), the sulphurous flavour increases in egg yolk from the fourth day of storage, concomitantly with emergence of the fish odour in egg white.

Appendix B. Detailed modelling results

In this Appendix more detailed information is provided about the modelling inputs and outputs for the MAESM model. Table B1 presents a summary of the information used in the model about the storage time and temperature of eggs destined for use in catering or food service and institutional settings.

Table B1: Summary of the information collected from industry experts regarding the time and temperature of storage of eggs in the EU for eggs used in catering/food service and institutional settings

Stage	Time (hours)			Temperature (°C)		
	Minimum	Most likely	Maximum	Minimum	Most likely	Maximum
On farm	0	45	168	4	15	30
Transport to grading	0	6	48	4	15	30
Grading	0	18	168	5	15	30
Transport to wholesale	0	5	48	0.1	14	30
Wholesale/ distribution centre	0	23	336	0.1	13	28
Transport to catering	0	7.5	36	0	14	30
Catering	0	7	27	0	8	28

The impact of prolonged storage of eggs can also be assessed by estimating the changes in concentration of *S. Enteritidis* in the egg. Figure B1 shows the distribution of the concentration in log₁₀ CFU/egg at the end of the household stage under the baseline scenario, i.e. under the current storage conditions (sell-by date of 21 days and best-before date of 28 days).

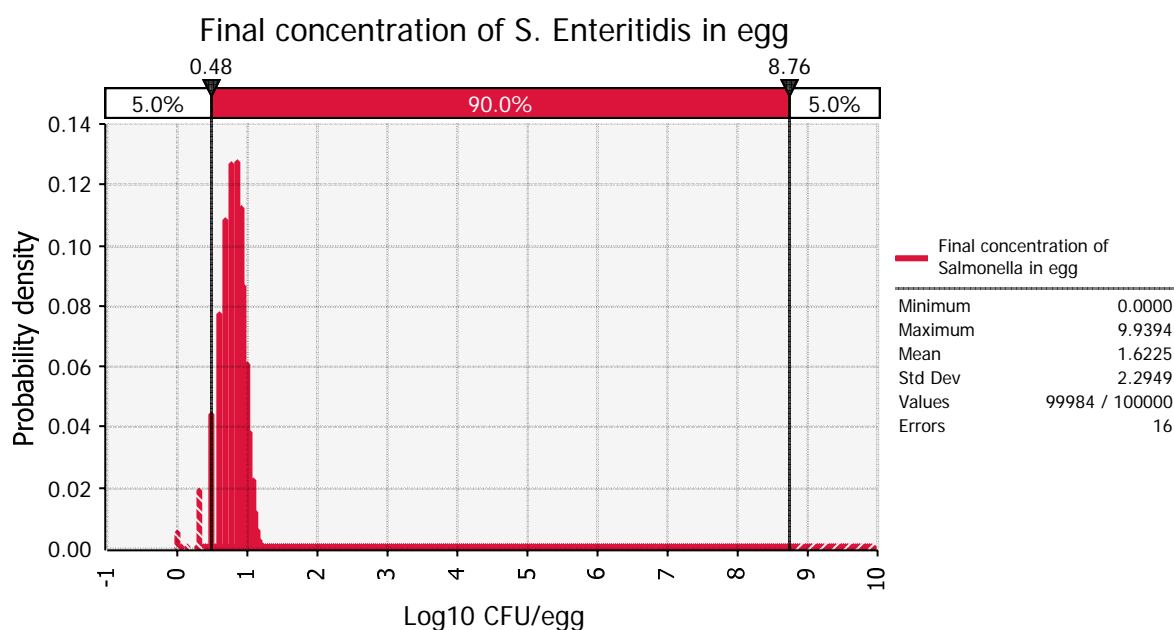


Figure B1: Distribution of the concentration of *S. Enteritidis* in log₁₀ CFU/egg at the end of the household stage under the baseline scenario, as estimated using the MAESM.

To illustrate the differences when prolonging the storage of the eggs, Figure B2 shows the distribution of the concentration in log₁₀ CFU/egg at the end of the household stage under the worst-case scenario considered in this assessment; a prolongation of the sell-by date to 42 days combined with a best-before date of 70 days. The impact is best observed in a much higher mean *S. Enteritidis* log₁₀ CFU/egg, 3.6 compared to 1.6 for the baseline scenario, as the shape of the distribution is similar.

Refrigerating during retail in this worst-case scenario would result in a lower mean, 2.2 \log_{10} CFU/egg, also with a similar shape of the distribution.

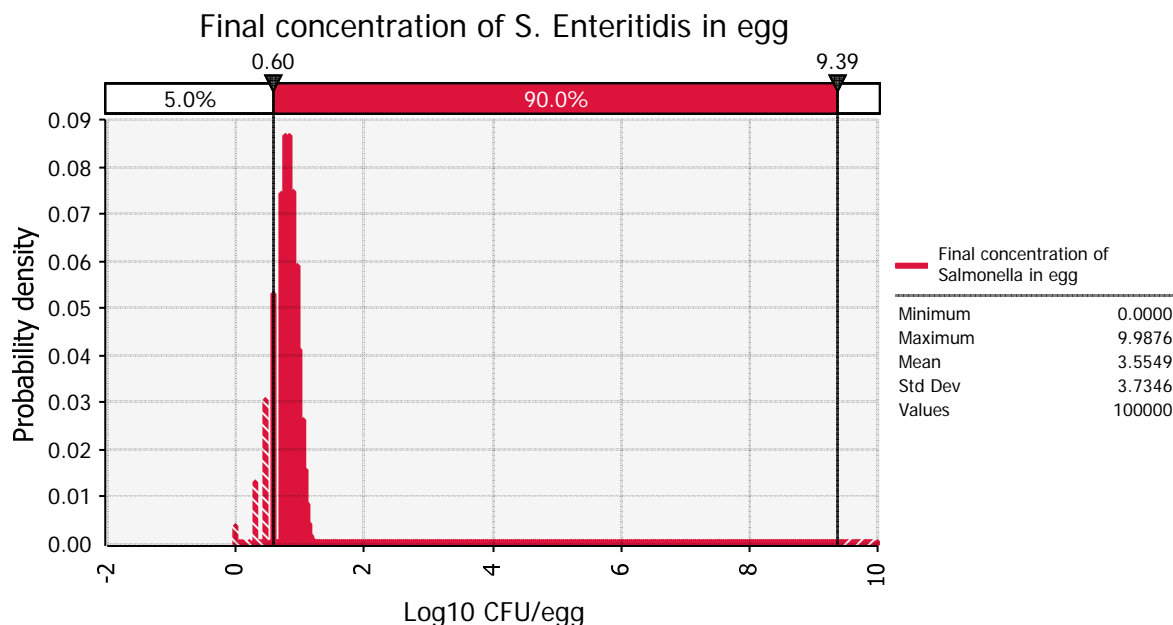


Figure B2: Distribution of the concentration of *S. Enteritidis* in \log_{10} CFU/egg at the end of the household stage under prolonged storage conditions, with a sell-by date of 42 days and a best-before date of 70 days, as estimated using the MAESM.

Taking the mean values for these concentrations found at the end of the household stage, the changes according to increasing best-before dates can be seen in Figure B3, for a sell-by date of 42 days and under the same storage conditions as those in the baseline scenario and with refrigeration at retail.

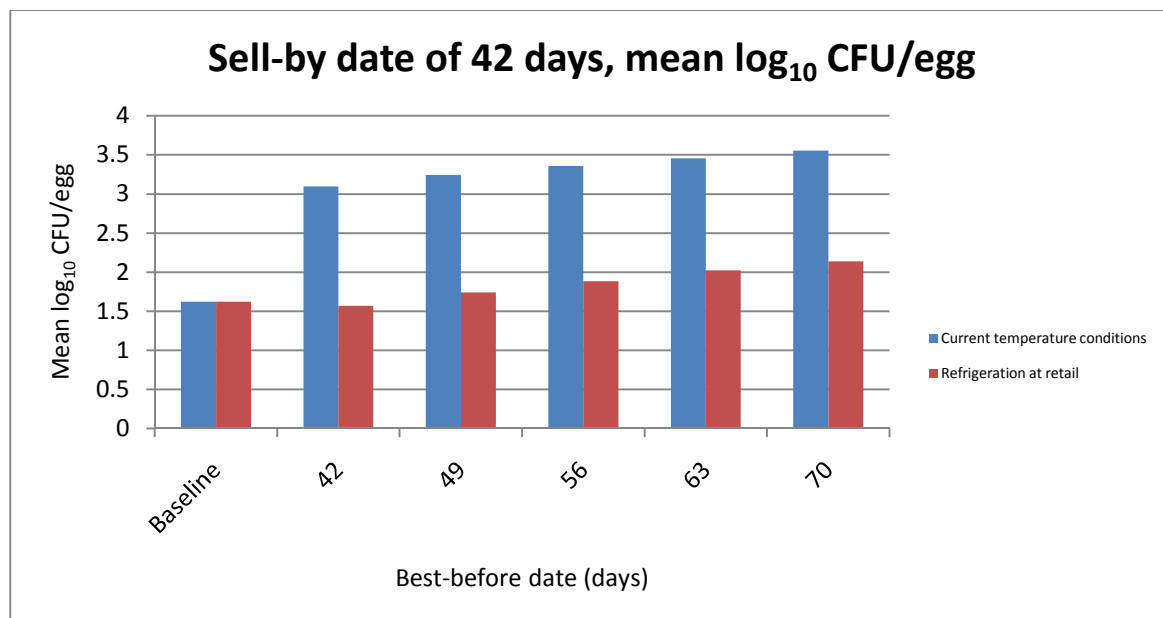


Figure B3: Changes in the mean concentration of *S. Enteritidis* in \log_{10} CFU/egg at the end of the household stage under prolonged storage conditions, with a sell-by date of 42 days and different best-before dates, as estimated using the MAESM.

Figure B4 shows the equivalent graph for a less extreme scenario, with a shorter sell-by date of 28 days and different best-before dates.

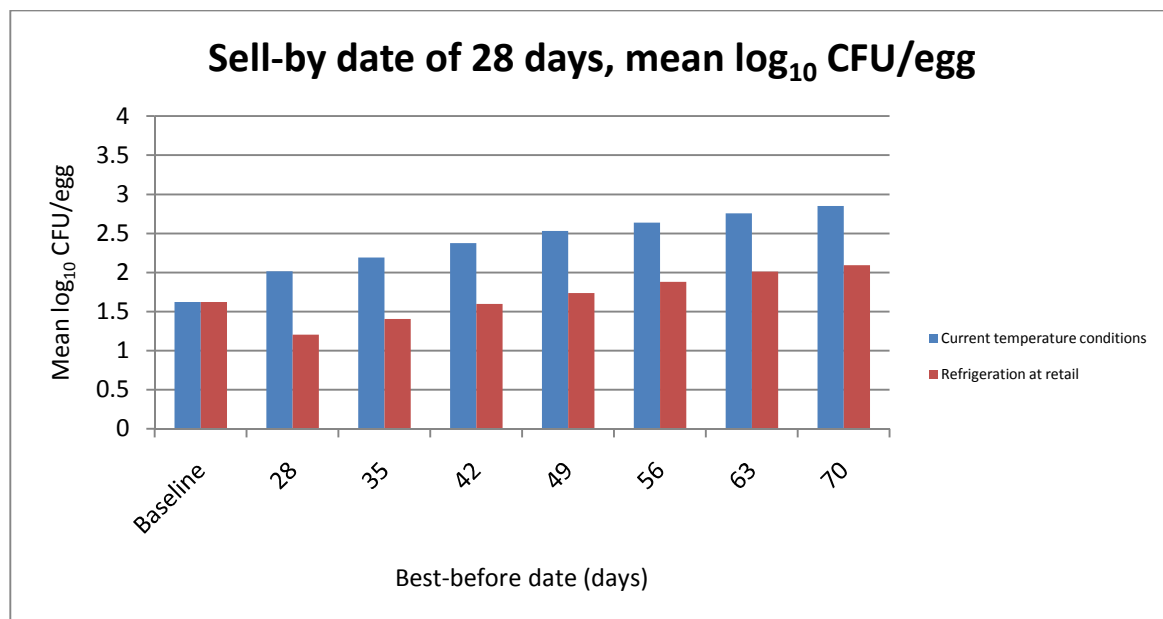


Figure B4: Changes in the mean concentration of *S. Enteritidis* in log₁₀ CFU/egg at the end of the household stage under prolonged storage conditions, with a sell-by date of 28 days and different best-before dates, as estimated using the MAESM.

Figure B5 shows the distribution for the probability of illness for uncooked egg meals in the baseline scenario, using contaminated eggs only (the absolute probability can be obtained by multiplying this by the rate of contaminated eggs – 0.04 % –, as explained in Section 13.2.1). The same distribution, but for the worst-case scenario mentioned above (a prolongation of the sell-by date to 42 days combined with a best-before date of 70 days) is shown in Figure B6.

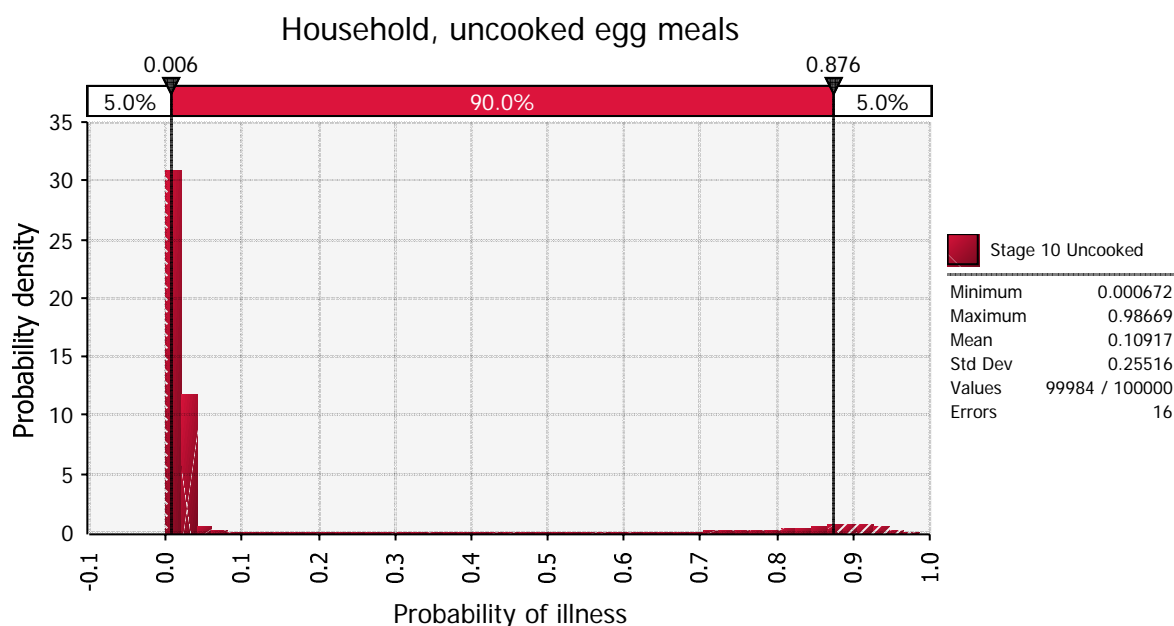


Figure B5: Probability of illness for uncooked egg meals in the baseline scenario (sell-by date of 21 days, best-before date of 28 days), estimated with the MAESM using contaminated eggs only

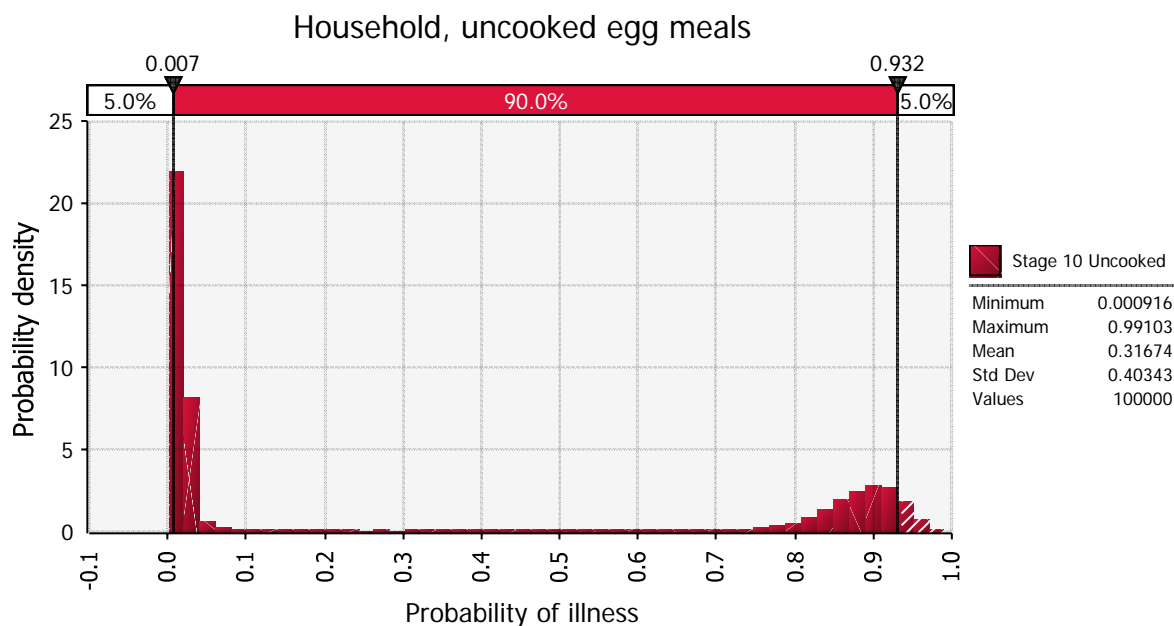


Figure B6: Probability of illness for uncooked egg meals in the worst-case scenario (sell-by date of 42 days, best-before date of 70 days), estimated with the MAESM using contaminated eggs only

The shape of the probability in these graphs resembles a bimodal distribution, with most of the values accumulated on the left hand side and some to the right (especially in the worst-case scenario). This is likely due to the combined effect of the yolk membrane breakdown time, which, if not reached, limits the growth of *S. Enteritidis* (values to the left of the graph) and the maximum number of *S. Enteritidis* that can grow in a single egg. This was set as a *Pert* distribution with values 10^8 , 10^9 and 10^{10} respectively for minimum, most likely and maximum \log_{10} CFU per egg, which would explain the bell shape of the right hand values in the graphs.

In Section 13.3 references have been made to the fact that the risk arising from well-cooked egg meals, whether from the baseline or alternative scenarios, is very small. To illustrate this, the figures below show the absolute risk estimates for the three preparation methods, comparing the baseline scenario with the worst-case scenario of a sell-by date of 42 days combined with several best-before dates. Figure B7 represents this scenario under current temperature conditions, and Figure B8 if refrigeration were implemented in retail establishments.

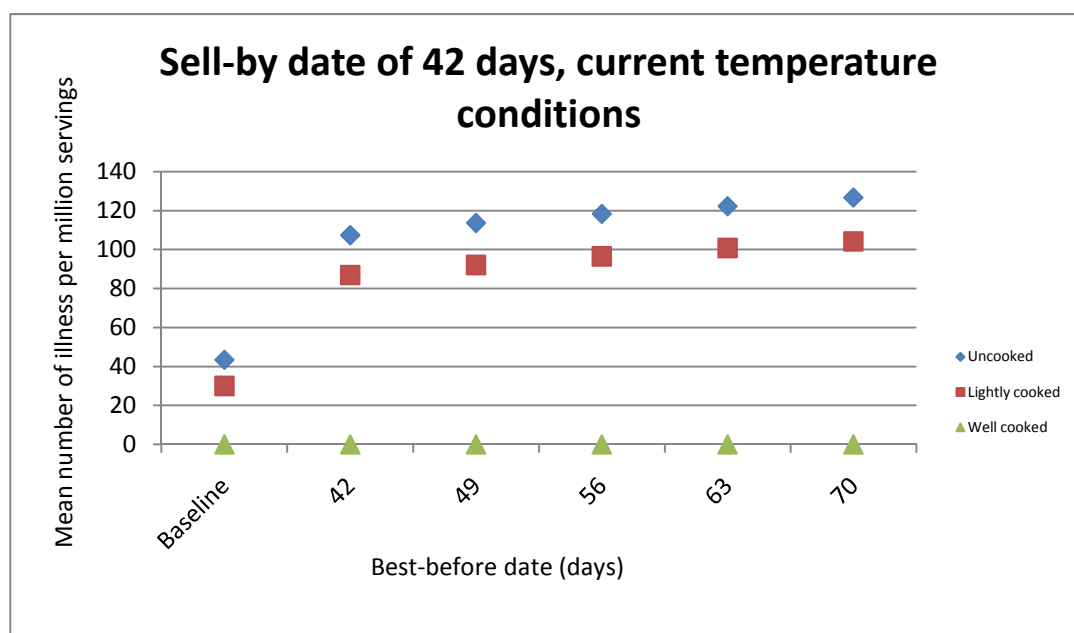


Figure B7: Changes in the mean number of illnesses per million servings at the end of the household stage under prolonged storage conditions, with a sell-by date of 42 days and different best-before dates, as estimated using the MAESM. Temperature of storage is the same as currently.

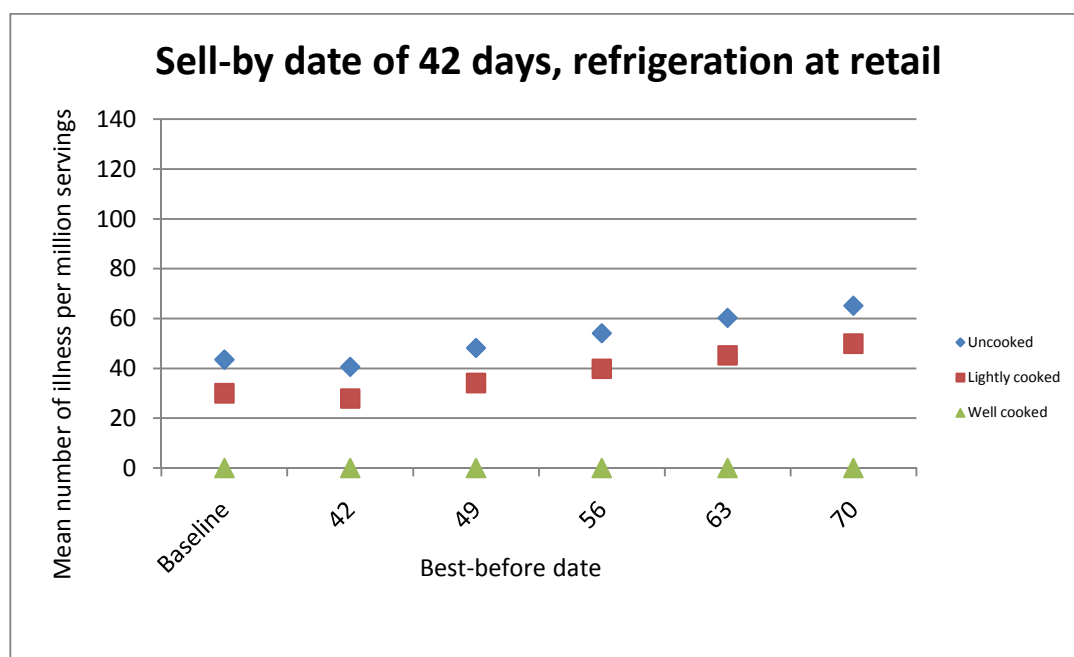


Figure B8: Changes in the mean number of illnesses per million servings at the end of the household stage under prolonged storage conditions, with a sell-by date of 42 days and different best-before dates, as estimated using the MAESM. Refrigerated temperature during retail stage.

Although the effect of refrigeration at the household was not explicitly assessed in this Opinion, Table B2 presents several extreme scenarios exploring the influence of both the absence and full implementation of refrigeration at that stage, for the worst-case scenario evaluated (i.e. sell-by date of 42 days and a best-before date of 70 days). Compared to the baseline scenario, the relative risk when implementing refrigeration in 100 % of the households is 2.0, while it is 2.9 when the proportion of households using refrigeration has a most likely value of 76 %. In addition, when refrigeration at retail is also implemented in 100 % of the establishments, these values are 0.4 and 1.5 respectively. It should

be noted that the observed influence of household refrigeration on the relative risk is related to the fact that in the worst case scenario a proportion of the eggs could spend up to four weeks at the household.

Table B2: Influence of household temperature (implemented in 100 % of households) in combination with temperature at retail on the risk arising from the consumption of eggs, for the baseline and worst-case scenarios

Scenario	Uncooked egg meals		Lightly cooked egg meals	
	Absolute risk, mean number of illnesses per million servings	Relative risk of illness	Absolute risk, mean number of illnesses per million servings	Relative risk of illness
Baseline scenario ¹	43.5	1.0	30.0	1.0
Worst-case scenario ² temperature conditions as in baseline scenario	126.7	2.9	104.2	3.5
Worst-case scenario ² , baseline temperature at retail, 100 % refrigeration at household	85.1	2.0	66.2	2.2
Worst-case scenario ² , 100 % refrigeration at retail, baseline temperature at the household	65.1	1.5	49.9	1.7
Worst-case scenario ² , 100 % refrigeration at retail, 100 % refrigeration at household	16.8	0.4	5.9	0.2

1: Baseline scenario: Sell-by date 21 days, best-before date 28 days. 15 % refrigeration at retail (most-likely); 76 % refrigeration in household (most-likely).

2: Worst-case scenario: Sell-by date 42 days, best-before date 70 days.

ABBREVIATIONS

a_w	Water activity
AECL	Australian Egg Corporation Limited
CDF	Cumulative density function
CI	Confidence interval
DALYs	Disability-adjusted life years
DT	Definitive phage (type)
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFSA BIOHAZ Panel	EFSA Panel on Biological Hazards
ELISA	Enzyme-linked immunosorbent assay
EPM	Egg Pooling Module
ESBLEC	Extended-spectrum beta-lactamase-producing <i>E. coli</i>
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FSIS	Food Safety and Inspection Service
Ig	Immunoglobulin
ISM	Inner shell membrane
MAESM	Modified Australian Eggs Storage Model
MS	Member State
OSM	Outer shell membrane
OVAX	Ovalbumin-related protein X
PCR	Polymerase chain reaction
PT	Phage type
SE	<i>Salmonella</i> Enteritidis
TESSy	The European Surveillance System
ToR	Term of Reference
TT-SAM	Turkey-Target <i>Salmonella</i> Attribution Model
USDA	United States Department of Agriculture
US FDA	United States Food and Drug Administration
VMOII	Vitelline membrane outer layer protection II
WHO	World Health Organization
YMT	Yolk membrane breakdown time